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BY

R. W. MARSH

AND

I. THOMAS

WITH THE ASSISTANCE OF

F. C. BAWDEN J. T. MARTIN
M. J. R. HEALY E. A. PARKIN
D. J. WATSON

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LABORATORY ERRORS ASSOCIATED WITH THE ESTIMATION OF THE POPULATION DENSITY OF *HETERODERA* SPECIES IN SOIL

BY F. MORIARTY

School of Agriculture, Cambridge

(With 4 Text-figures)

As unexpectedly large sampling errors were obtained in preliminary population studies on *Heterodera*, an examination was made of the laboratory errors associated with sampling for *H. göttingiana* and *H. schachtii*. Soil samples were taken from microplots and appeared to be satisfactory, being without the usual heterogeneity found in sampling from fields. There was little evidence of errors introduced by the technique used for estimating the number of eggs and larvae in a subsample. These errors, when they occurred, were always small, and this standard technique was deemed satisfactory. The logarithmic transformation was suitable for statistical analysis of both cyst and egg counts.

Apart from the residual variation, there were two other important sources of variation when sampling for eggs. There was a large variation between subsamples, caused by differences in the number of cysts and in the number of eggs per cyst. There was also in some instances a difference between observers counting the same eggs of up to 20 %. Where a comparative measure of density is sufficient, this bias is probably unimportant, and to increase accuracy of estimates of eelworm density, most laboratory work should be devoted to the separation of cysts from the soil sample.

INTRODUCTION

There is an extensive literature of population studies on eelworms of the genus *Heterodera*, but the few investigations into the errors associated with sampling were inadequate to assess their magnitude. For the work now described pea root eelworm (*H. göttingiana* Liebscher) and beet eelworm (*H. schachtii* Schm.) were used, but the results should be relevant to other *Heterodera* species. The main purpose was to study the errors associated with estimating the density of eggs and larvae in the soil, and secondarily the errors associated with estimating the density of cysts.

METHODS

Standard methods were used for obtaining soil samples and for estimation of their cysts and eggs. Soil samples were taken from microplots: experimental plots of 0·8 sq.yd. separated by concrete slabs (Jones, 1956). Each sample consisted of twenty uniformly spaced borings taken to a depth of 8 in. with a $\frac{3}{4}$ in. diameter semi-cylindrical sampler. They were then sieved, air-dried and thoroughly mixed. Cysts were collected by flotation and decanting from conical 1 l. flasks; the cysts were squashed, and aliquots from the suspension placed into counting slides (Williams &

Winslow, 1955). The slides were examined under a stereoscopic microscope with × 25 magnification.

Throughout this paper, the term sample is used for the mass of soil taken from a microplot and treated as one unit, from which the density of eelworms in the microplot is estimated. The term subsample indicates the quantity of soil that is taken from a sample and placed in one flask for the removal of cysts by flotation.

Three observers were concerned with this work, and are denoted by the letters A, B and C.

SAMPLING ERRORS WITH PEA ROOT EELWORM

Eight samples were taken from one microplot containing a light sandy soil from Aldringham, Suffolk, infested with *H. göttingiana*. This soil had been fallow, free of weeds, from July 1955, when a main crop of Onward peas was lifted, until the time that these samples were taken, in June 1958.

Varying numbers and weights of subsamples were taken, and are shown in Table 1, together with details of egg suspension volume and number of aliquots taken. The cysts from 200 g. subsamples were obtained from pairs of 100 g. subsamples, which was considered to be the maximum weight suitable for the flotation process. All counts of the eggs and larvae were made by observer B.

The volume in which the eggs and larvae were suspended was adjusted so that the numbers per square of the counting slide should not exceed 100, as above this density it is difficult to ensure that each individual is counted once and once only. Suspensions in 33·25 ml. were agitated by bubbling air for about 10 sec. through a pipette into the bottom of the tube, immediately before transferring an aliquot to the counting slide. Larger volumes were agitated by a vibratory stirrer. In all instances the number of eggs and larvae occurring in each of the four corner squares of the counting chamber was recorded. This standard arrangement was preferred to a random one because there was no apparent bias between squares, it was desirable to have conditions of counting as standardized as possible, and the normal steady routine of counting might be upset by use of continually changing patterns of squares. With suspensions of 33·25 ml. there was insufficient liquid for more than three aliquots. When suspensions were made up to 66·5 ml., the first three aliquots were examined; when suspensions were made up to 133 ml., the first six aliquots were examined. The counting slide contained four chambers, of which the first three were used, always in the same order. A random distribution of aliquots between chambers was tried, but in practice, after examining suspensions from several subsamples, errors of mistaken arrangement occurred: this was considered more serious than the confounding of possible between-aliquot effects with their arrangement in the counting slide. With suspensions of 133 ml., the fourth aliquot was placed in the same chamber as the first aliquot, and so on.

The data were first examined to determine the relationship between the mean number of eggs and larvae per square and the variance. The subsamples within each sample were arranged in increasing order of total number of eggs and larvae counted and then grouped into blocks. Analyses of variance on all counts for each

TABLE 1. *Summary of samples of* Heterodera göttingiana *taken from one Aldringham microplot in* 1958

	Sample							
	A/1*a*	*A*/1*b*	*A*/2	*A*/3	*A*/4	*A*/5	*A*/6	*A*/7, 8
Observer collecting cysts	A	A	B	B	A	B	A	A and B
Weight of subsamples (g.)	10	25	50	100	100	2 × 100	2 × 100	2 × 100
Number of subsamples	20	20	20	9	9	5	5	9
Number of aliquots examined in each suspension	3	3	3	3	3	6	6	6
Volume of egg suspension (ml.)	33·25	33·25	33·25	66·5	66·5	133	133	133
Proportion of suspension above one square of counting chamber	1/100	1/100	1/100	1/200	1/200	1/400	1/400	1/400
Average number of eggs and larvae/square of counting chamber	13·9	32·5	69·3	70·8	70·4	66·7	71·2	65·3
Proportion of total suspension examined	12/100	12/100	12/100	6/100	6/100	6/100	6/100	6/100

TABLE 2. *Summary of analyses of variance performed on egg and larval counts from eight groups of subsamples, using the logarithmic transformation*

S = squares within aliquots, A = aliquots, U = subsamples.

	Source of variance	Sample							
		A/1*a*	*A*/1*b*	*A*/2	*A*/3	*A*/4	*A*/5	*A*/6	*A*/7, 8
D.F.	S	9	9	9	9	9	23	23	23
	A	2	2	2	2	2			
	U	19	19	19	8	8	4	4	8
	Residual	209	209	209	88	88	92	92	184
M.S.	S	0·0243	0·0031	0·0067	0·0061	0·0039	0·0057	0·0033	0·0051
	A	0·0499	0·0836	0·0335	0·0033	0·0051			
	U	0·0908	0·1643	0·0206	0·0348	0·0190	0·0791	0·0632	0·0257
	Residual	0·0153	0·0073	0·0033	0·0039	0·0035	0·0055	0·0054	0·0048
Components of variance	S	—	—	0·0001	—	—	—	—	—
	A	0·0004	0·0010	0·0003	—	—	—	—	—
	U	0·0063	0·0131	0·0014	0·0026	0·0013	0·0031	0·0024	0·0009
	Residual	0·0153	0·0073	0·0033	0·0039	0·0035	0·0055	0·0054	0·0048

sample were then made using the original data, and also with square root and logarithmic transformations. The residual mean squares showed clearly that the original data were unsatisfactory for statistical analysis, for in general the variance increased with the count. The relationship between the residual standard deviation and the mean is shown in Fig. 1. There is little to choose between the square root and logarithmic transformations, although the latter is slightly to be preferred. Histograms of the residuals were made, which showed little skewness with any of the three forms of the data, but there was less spread with the logarithmic transformation. For further analysis of these results, the logarithmic transformation was used.

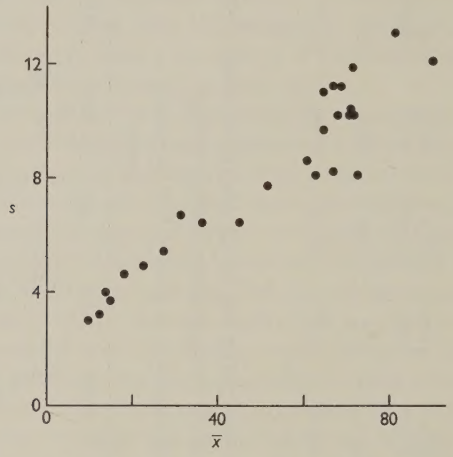

Fig. 1. The standard deviation (s) and the mean ($\bar{x}$) for counts from egg suspensions of *Heterodera göttingiana*.

An analysis of variance was made to determine the significance of variation between subsamples (U), variation between aliquots related to their order (A), and variation between squares within aliquots (S). In no analysis was the U × A interaction significant, and it was pooled with the U × S interaction to form the residual variance. For those samples with six aliquots per subsample, the breakdown of variance is more complicated, because the first and second sets of three aliquots were counted in the same chambers. The results, together with the values of the variances where significant at the 0·05 probability level, are shown in Table 2. Further analysis was made of the variance due to squares with six aliquots per subsample, when variance between sets of squares was compared with that within aliquots, and variance between replicates with that due to the interaction of replicates with squares within aliquots. In no instance was a significant result obtained at the 0·05 probability level.

Except for $A/2$, there is no significant variation within aliquots, which suggests that the distribution of eggs and larvae within counting chambers is not too far

from random. In all three samples where the suspensions were agitated by blowing air through a pipette (*A*/1*a*, *A*/1*b* and *A*/2) there is significant variation between aliquots: the later aliquots have fewer eggs and larvae than the earlier ones. There

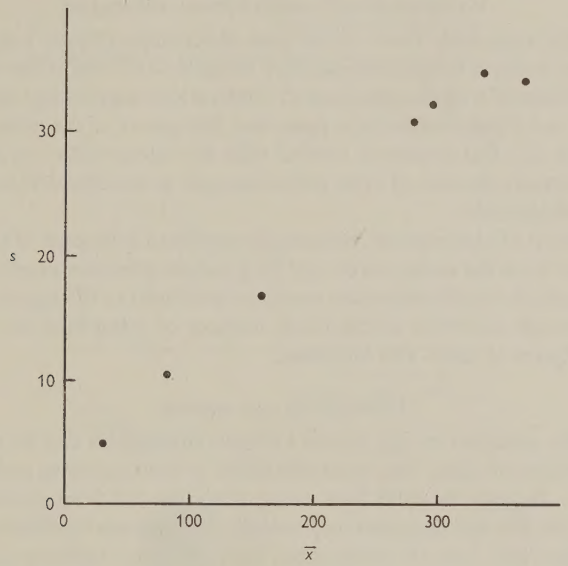

Fig. 2. The standard deviation (s) and the mean ($\bar{x}$) for cyst counts of *Heterodera göttingiana* from subsamples of different weights.

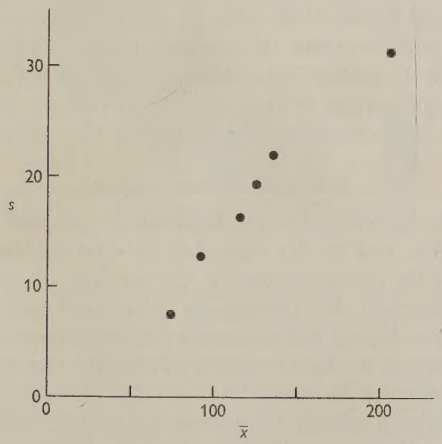

Fig. 3. The standard deviation (s) and the mean ($\bar{x}$) for cyst counts of *Heterodera göttingiana* from batches of 50 g. subsamples from different Aldringham microplots.

was no significant variation between aliquots in the other samples, where suspensions were agitated automatically. With all samples variation between subsamples was significant at the 0·001 level.

Variation in cyst counts between subsamples

It is usually supposed, from theory and observation (Jones, 1945), that cyst counts from successive subsamples conform roughly to a Poisson distribution. The cyst counts obtained with the above set of observations suggest that the variance is proportional to a value between the mean and the square of the mean, but nearer the latter (Fig. 2). But caution is needed with the interpretation of these results, because increase in number of cysts per subsample is associated with an increase in weight of subsample.

In a further set of observations, one sample was taken from each of six microplots containing soil from the same source, and 50 g. subsamples examined by observer B for cysts. Again the variance is more nearly proportional to the square of the mean (Fig. 3), although this time as the mean number of cysts increases the ratio of variance to square of mean also increases.

Variation in cyst contents

Some of the variation in egg counts between subsamples can be related to the different numbers of cysts. But cysts also differ in their contents, and this too may be important. In 1957 the cysts from 100 g. of similar soil from another microplot were graded for size and examined individually for contents by observer C (Fig. 4). This population had cysts of various ages, for host plants had been grown for the previous 4 years, since the soil was first obtained. Compared with the population studied by Winslow (1955*a*), the cysts were smaller, but again those cysts that passed through a 30-mesh sieve and were retained by a 60-mesh sieve contained most of the eggs and larvae. With cysts of all sizes, a large proportion had no contents. Of those with contents, the average number of eggs per cyst increased with cyst size. From the bottom histogram it can be seen that a relatively few cysts contributed a large proportion of the total number of eggs. For example, in this sample 12% of the cysts contained over 40% of all the eggs and larvae.

Variation between samples

Two *t*-tests made by comparing the logarithm of the cyst counts obtained in sample *A*/3 with *A*/5, and in *A*/4 with *A*/6, gave no evidence of heterogeneity between samples of the cyst population in the top 8 in. of soil in a microplot as estimated by one observer. But comparison of the combined cyst counts for *A*/3 and *A*/5 with those for *A*/4 and *A*/6 indicated a definite difference between observers A and B, with B having the higher counts (Table 3). The egg counts from suspensions of all four samples were made by observer B. If B picks more cysts than A, and there is no difference between the average egg content per cyst of either group of cysts, then there should be a difference between the egg counts made by one observer from the two groups of cysts. There was no such difference (Table 1).

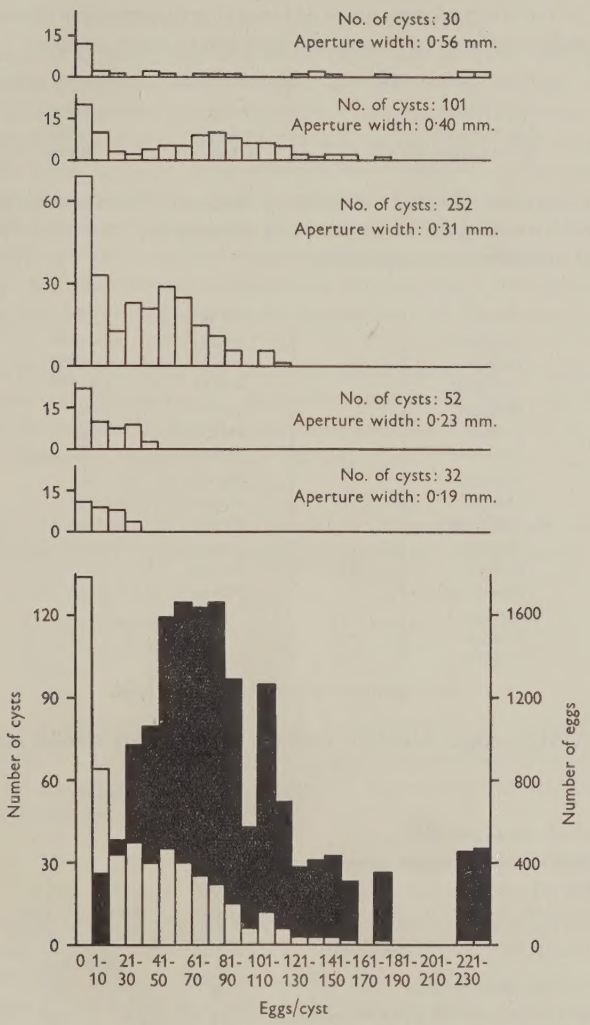

Fig. 4. Histogram of the frequency distribution of eggs per cyst for *Heterodera göttingiana*. The cysts from 100 g. of soil were washed through a series of sieves of decreasing aperture size. The number of cysts and size of sieve are shown on the top five histograms. No cysts were retained on a 20-mesh (0·90 mm. aperture width) sieve. The bottom histogram shows the results for all cysts, together with the total number of eggs for each group of cysts (blocked-in). 0·56 aperture = 30 mesh, 0·40 aperture = 40 mesh, 0·31 aperture = 50 mesh, 0·23 aperture = 60 mesh, 0·19 aperture = 80 mesh.

TABLE 3. *Variation of cyst counts of* Heterodera göttingiana *between samples and observers, using the logarithmic transformation*

Samples being compared	Mean difference	Standard error
$A/3$ with $A/5$	0·043	0·021
$A/4$ with $A/6$	0·019	0·023
$A/3+5$ with $A/4+6$	0·091	0·014

TABLE 4. Heterodera schachtii: *summary of analyses of variance performed on egg and larval counts from three groups of subsamples, each with three aliquots, using the logarithmic transformation*

Source of variance	Soil	D.F.	M.S.	F	Components of variance
U	Isleham	5	0·3205	31·73***	0·0026
	Oxlode		0·1051	6·22***	0·0074
	Spalding		0·1412	5·79***	0·0097
S	Isleham	9	0·0180	1·78	—
	Oxlode		0·0175	1·04	—
	Spalding		0·0210	—	—
A	Isleham	2	0·0001	—	—
	Oxlode		0·0279	1·65	—
	Spalding		0·0211	—	—
Residual	Isleham	55	0·0101	—	0·0101
	Oxlode		0·0169	—	0·0169
	Spalding		0·0244	—	0·0244
Total	Isleham	71	—	—	—
	Oxlode		—	—	—
	Spalding		—	—	—

*** Significant at 0·001 probability level.

TABLE 5. Heterodera schachtii: *variance of cyst counts between subsamples, all with* 5 D.F.

	Isleham soil			
Weight of subsamples (g.)	10	25	50	100
Average number of cysts per subsample ($\bar{x}$)	23	48	88	198
Variance (s^2)	25·0	31·8	105·8	575·6
$s^2/\bar{x}$	1·09	0·66	1·20	2·91
$s/\bar{x}$	0·217	0·118	0·117	0·121
	Oxlode soil			
Weight of subsamples (g.)	10	25	50	100
Average number of cysts per subsample ($\bar{x}$)	25	42	142	287
Variance (s^2)	35·4	34·6	51·8	465·6
$s^2/\bar{x}$	1·42	0·82	0·36	1·62
$s/\bar{x}$	0·238	0·140	0·051	0·075
	Spalding soil			
Weight of subsamples (g.)	10	25	50	100
Average number of cysts per subsample ($\bar{x}$)	11	19	37	75
Variance (s^2)	31·4	17·0	22·6	160·4
$s^2/\bar{x}$	2·85	0·89	0·61	2·14
$s/\bar{x}$	0·509	0·217	0·128	0·169

Sampling errors with beet eelworm

One large sample of sixty borings was taken from each of three microplots, each with a different soil infested with beet eelworm. Six 10, 25, 50, and 100 g. subsamples were taken from each sample, and the cysts counted. The cysts from each of the 50 g. subsamples were squashed and made up into a suspension of 33·25 ml. Twelve counts were made in three aliquots, as described earlier for pea root eelworm, except that the aliquots were distributed randomly in the counting slide. Analyses of variance were made on the logarithms of the egg counts from each set of subsamples (Table 4). A similar result to that shown in Table 2 was obtained, with significant variation between subsamples. The average count per square was too uniformly low to provide any useful information on the change of variance with mean.

Compared with the results for pea root eelworm, variance of cyst counts between subsamples gave less definite results, due partly perhaps to the smaller number of counts (Table 5). In general, variance increased with the mean, with the exception of the 10 g. subsamples, whose variance was large.

Table 6. Heterodera schachtii: *variance of cyst counts between samples from one Oxlode microplot, using the logarithmic transformation*

Source of variance	D.F.	M.S.	*F*
Between samples	6	0·0033	1·03
Between subsamples	35	0·0032	—
Total	41	—	—

From each of another seven samples, each of twenty borings, all taken from one Oxlode microplot, six 50 g. subsamples were taken . An analysis of variance on the logarithm of the cyst counts (Table 6), suggested absence of heterogeneity between samples, as with pea root eelworm.

Observer variation

During November 1955, one sample was taken from each of thirty-two microplots containing the soil from Aldringham, infested with *H. göttingiana*, that was used in observations already described. Two 50 g. subsamples were taken from each. Observers A and B took one of each pair of subsamples at random, prepared a suspension and transferred an aliquot to a counting chamber. Sufficient squares of the counting chamber were examined to obtain a total count of at least one hundred eggs and larvae. Usually four suspensions were prepared at a time. After counting, the observers exchanged counting slides, and made counts on the other observer's suspensions. All counts were expressed per single square, transformed to logarithms, and a split-plot analysis made. A similar series of observations and analysis was made in November 1957 by observers A and C on thirty-nine microplots containing soil from Spalding infested with *H. schachtii*.

There was no significant difference in either instance for the counts obtained by the two observers, although in 1957 the interaction of source of cysts (C) and observer counting eggs (O) was significant ($F = 7{\cdot}43^{**}$, $n_1 = 1$, $n_2 = 76$). The mean counts are shown in Table 7.

Observers A and B again estimated the population in some of the Aldringham microplots in December 1958. One sample was taken from each of thirty-three microplots, and four subsamples were taken from each sample. The observers took one of the first pair and one of the second pair of subsamples randomly, and obtained an aliquot for egg counting. Both observers counted their own and the other's suspensions as before. The counts were divided into four groups (of thirteen, six, seven and seven samples), according to the days on which they were made. A split-plot analysis was made; none of the interactions within plots was significant. The mean square for observers was tested against the combined mean square of the subplot residual and the two second-order interactions containing the factor *M* (between microplots). Within all four groups there was a significant difference between observers (Table 8); further analysis showed no significant change in this observer variation between groups.

TABLE 7. *Comparison of counts made by two observers on the same eggs and larvae in counting slides*

Series of observations	Group	Mean number eggs and larvae/square (calculated from log. transformation) counted by observer			Mean count by 2nd observer as % of mean count by A	Log. mean difference and standard error between counts by two observers
		A	B	C		
Aldringham, 1955		64·6	64·3	—	100	0·002 ± 0·008
Spalding, 1957		56·8	—	56·9	100	0·001 ± 0·006
Aldringham, 1958	1st group counted 12 December	64·4	74·6	—	116	0·074 ± 0·009
	2nd group counted 15 December	80·2	88·9	—	111	0·045 ± 0·009
	3rd group counted 19 December	72·1	80·4	—	116	0·047 ± 0·017
	4th group counted 22 December	71·0	80·0	—	113	0·052 ± 0·012
Spalding, 1959	1st group counted 27 February	10·1	10·9	—	108	0·033 ± 0·011
	2nd group counted 5 March	8·9	9·9	—	111	0·046 ± 0·011
	3rd group counted 7 March	10·6	12·9	—	122	0·085 ± 0·009

In February 1959, observers A and B made counts on one sample from each of forty Spalding microplots. This time two subsamples were taken from each sample, and they were treated as before. The counts were in three groups (of fourteen, fourteen and twelve samples), according to the days on which they were made. Again there was a significant difference between observers in all three groups, and

also there was a significant change between groups (Table 7). In the second group there was a significant difference according to the source of cysts (0·01 probability level) and in the third group the C × O interaction was significant at the 0·05 probability level.

TABLE 8. *Analysis of variance for the logarithm of the egg counts in the Aldringham* 1958 *series*

Group	Source of variance	D.F.	M.S.	*F*
1st	Between observers	1	0·1085	51·67***
	Residual	36	0·0021	
2nd	Between observers	1	0·0239	23·90***
	Residual	15	0·0010	
3rd	Between observers	1	0·0321	9·73**
	Residual	18	0·0033	
4th	Between observers	1	0·0375	18·85***
	Residual	18	0·0020	

** Significant at 0·01 probability level; *** at 0·001 probability level.

DISCUSSION

It has been realized for a long time that when screening possible nematicides the examination of eelworms after treatment may not be a reliable guide to treatment effects. Some form of bioassay is commonly used. Counts of viable eggs and larvae are used for population studies on *Heterodera* species, and several authors have commented on the difficulties of distinguishing between live and dead eelworms (Jones, 1955). The usual criteria are shape and coloration, and it is generally supposed that with trained observers these are satisfactory.

The results in this paper show that observer variation can be important. In 1955 the eelworm population in the Aldringham microplots was young (for previous history see Jones & Moriarty (1956)): there was no significant difference between observers A and B (Table 7). By 1958, after 3 years with various combinations of fallow, non-host crops, and host crop failures, the population was much older: there was a difference between A and B of more than 10 %. This change is not necessarily caused by the older population: it could be a change with time between A and B. The analysis of the Spalding 1959 counts showed that two observers' standards can change relative to each other within a few days (Table 7). At least one observer's standards for determining viable eggs and larvae must have altered. Even if an observer is consistent, it is very difficult to compare his counts with an objective standard. Jones (1945) gives figures for counts by pairs of observers of cysts, viable cysts, and eggs, obtained from pairs of soil samples. It is not possible to compare the observer difference for egg counts with those obtained in this work because the observers counted different samples.

This problem does not seem very important with young populations, but with old populations observers often experience great difficulty in deciding whether or not to include eggs and larvae in their counts. It is of interest therefore that the standard deviation for the difference between observers' counts of the same eggs

and larvae seems independent of the age of the population (Table 7). It is also large. Sometimes there is a difference between observers for cyst counts, but this is much less important. It is caused by differences in counting of old decaying cysts, which contain few eggs and do not materially affect the estimation of egg density (see *A*/3–6, Tables 1, 3).

For experiments on differences in eelworm density, resulting from various treatments, a relative measure is often sufficient. Then, provided that counts are proportional to the number of live eelworms, and with suitably arranged sampling, this bias is unimportant. But for experiments or observations where the actual density is needed, it can be serious. Winslow (1955*c*), when using *Solanum nigrum* L. as a trap crop for *Heterodera rostochiensis*, obtained a greater reduction of hatchable larvae than of the number of apparently viable eggs and larvae; he suggested that some of the cyst contents were either non-hatchable or non-viable. Similar results were obtained with *Matthiola incana* (L.) R.Br. as a trap crop for *Heterodera schachtii*. There have been some attempts to distinguish between live and dead eelworms by staining, but none has been much used. Boyd (1941), working with *H. rostochiensis*, used an aqueous solution of iodine and potassium iodide, but it has two disadvantages: the staining solution is itself toxic, and eelworms do not stain immediately after death. Homeyer (1953*a*, *b*) found a difference between live and dead eelworms for some species of *Ditylenchus* and *Aphelenchoides* stained with acridine orange. Budzier (1954) and van der Laan & Bijloo (1955) found it unreliable for larvae of *Heterodera rostochiensis* killed by chemicals. Doliwa (1956) found that dead larvae of *H. rostochiensis* were stained a diffuse yellow by a solution of chrysoidin, whilst live larvae were virtually unstained except for orange-coloured granules in the gut. This test was effective for larvae killed by various chemicals. Kämpfe (1956) confirmed and extended this work.

Apart from this bias, any estimate of eelworm density is subject to sampling errors. These can be divided into field errors, concerned with taking the sample, and laboratory errors, concerned with its subsequent treatment. The little available information about field errors deals mainly with cyst counts, but is sufficient to show that distribution in the field can be very patchy (Anscombe, 1950; Church, Gough & Southey, 1959; Fenwick, 1959; Fidler, Church & Southey, 1959). Each instance must be carefully investigated if knowledge of the cyst distribution is needed. With microplots, standard samples that consist of twenty borings reduce this error to a negligible size (Tables 1, 3 and 6). A detail of nomenclature might be noted here: since the borings taken from a microplot are based on a regular pattern, it is preferable to consider all the borings together as one sample (Finney, 1947), and not as a bulk sample such as is taken in field work (Anscombe, 1950).

Given perfect laboratory technique, cyst counts from subsamples of equal weight would conform to a Poisson distribution. Similarly, egg counts from suspensions prepared from these cysts would conform to a Poisson distribution (Peters, 1941). Jones (1945) examined ten 50 g. subsamples from each of twenty-eight bulk samples for cysts of *H. schachtii*. His technique was similar to that used for this work; he concluded that there was reasonable agreement with the Poisson

series, with high cyst counts deviating more markedly than low counts. However, for half of these samples the mean count was less than forty; in nine of the fourteen samples with higher mean counts, the variance was significantly greater (at the 0·05 probability level) than theoretically expected, using the χ^2 test. The χ^2 test is relatively insensitive with low numbers, and it is doubtful whether these figures do support the hypothesis of reasonable agreement with a Poisson distribution. The Fenwick can is commonly used for large quantities of soil (Goodey, 1957); the magnitude of the errors associated with it is uncertain. Fenwick (1940), in tests on the original version with ten subsamples containing *H. rostochiensis*, obtained a variance of 94 with a mean count of 1759 cysts. This indicates a far greater degree of uniformity in cyst distribution than would be expected by a random arrangement. Fidler *et al.* (1959), in a survey of this method with *H. major*, found that variance in cyst counts between subsamples exceeded that for a Poisson distribution. There was also a difference between observers, which could be due to differences in counting cysts, or of differences in the method of recovering cysts. The present results support the general conclusion that the variance of cyst counts is greater than might be expected from a Poisson distribution (Figs. 2, 3; Table 5). These counts are between a Poisson and lognormal distribution, and somewhat nearer to the latter. Sometimes observers obtain counts from successive subsamples with a variance smaller than that expected for a Poisson distribution, due to a subconscious attempt to make counts agree (Anscombe, 1950).

Jones (1945) used the same principle for estimating egg density of *H. schachtii* as that used in this work, but the methods differed considerably in detail. He found a tendency for successive counts to have a larger variance than would be expected for a Poisson distribution. This he ascribed to errors of counting, and the adhesion of eggs in twos and threes. The present results for *H. göttingiana* also show a greater variance than Poisson, which is more noticeable with the higher counts. Clumping of eggs did not appear to be very important with these particular samples. Bijloo (1954) found that eggs adhering to pieces of the cyst wall in *H. rostochiensis* caused a considerable error, and suggested the use of a homogenizer. This was confirmed by Reid (1955). There was no such difficulty in the present work. The general technique for sampling from an egg suspension appears satisfactory: even where there is a significant difference between aliquots or squares, the magnitude of the variance is relatively small (Table 2). The deviation from a Poisson distribution for egg counts is probably due to difficulties of counting. As with cyst counts, the logarithmic transformation seems most suitable. Peters (1948), in pot tests on *H. rostochiensis*, counted larvae hatched by Fenwick's hypochlorite technique. He found that the mean was proportional to a value between the standard deviation and the variance, and decided on the logarithmic transformation as most suitable for statistical treatment of the data. Fenwick (1942), in his description of this technique, obtained a Poisson distribution, but the mean count was low, about 10. With this method, there should be no difficulties of counting. Further observations by Peters & Fenwick (1949) showed that this method gives no indication of viability, and may not even give reliable counts of the total number of eggs and larvae.

To assess the accuracy of an estimate for egg density, given a representative soil sample, and knowing the most suitable transformation for the data, estimates of the variance within and between subsamples are also needed. The number of eggs and larvae per cyst was extremely variable in the Aldringham population of *H. göttingiana* (Fig. 4). Hesling (1959) obtained a similar frequency distribution for the cyst contents of *H. rostochiensis*, and Fenwick (1959) states that the standard deviation for counts of larvae within cysts is normally of the same order as the mean. This is reflected in the magnitude of the variance for egg counts between subsamples (Tables 2, 4). In all three populations of *H. schachtii*, and in all except one of the samples of *H. göttingiana*, the residual variance was larger, but the variance between subsamples is very important. For practical purposes the variance due to squares and aliquots, where significant, is so small that it can be ignored. The total variance for an estimate of the logarithm of the egg count from a soil sample then becomes

$$\frac{s^2}{ab}+\frac{s_u^2}{b},$$

where s^2 is the residual variance, s_u^2 the variance between subsamples, a the number of counts per subsample, and b the number of subsamples. To reduce the standard error, the number of subsamples should be increased because this affects both terms, whereas increasing the number of counts within subsamples only affects one term. Unfortunately, because of the labour in separating cysts from debris, it is much more arduous to increase the number of subsamples than to increase the counts per subsample. It is difficult to make a general comparison of the relative amount of labour involved in the counting of cysts and eggs, because the former is affected enormously by the amount of soil debris, and both are affected greatly by the age of the cysts. It is a simple matter to count the live eggs from the current season's cysts, but very tiring to count the live eggs from cysts 5 years old or more.

Counts of eggs and cysts are normally expressed as densities, for example, eggs per g. of soil. The multiplication factor used to obtain the density of eggs from the number of eggs counted depends on the weight of the subsample and the proportion of the egg suspension that is examined. With the logarithmic transformation, this multiplication factor will not affect the estimate of variance at all. The standard errors for the mean egg counts obtained in this work are shown in Table 9.

Most of the earlier population studies on *Heterodera* species were based on cyst counts, using Morgan's (1925) flotation technique. This was an inadequate measure (e.g. Smith & Prentice, 1929). Fenwick (1942) examined the problem and concluded that one should measure the density of larvae capable 'under theoretically ideal conditions' of entering host roots. Jones (1945) had also concluded independently that the important factor is the 'infestation potential', which can be estimated approximately by sampling for the density of viable eggs and larvae.

Normally a technique similar to that for this work is used. There are several variations (Goodey, 1957), but in all the distinction between 'viable' and 'non-viable' is based on the appearance of the eggs and larvae. In field survey and advisory work, estimates of eelworm density are often based on some classification of cysts

by their contents, such as 'full', 'half-full' and empty. But all these methods have two disadvantages. There is the subjective element in deciding whether to count an individual egg or larva, and it is doubtful whether all the live eggs and larvae can invade a host plant with equal facility. These considerations become more important with older populations, and with work where a relative measure of change is insufficient. Sometimes hatching tests are used in population studies, but so far only as a supporting measure to the above method. Also, these are not applicable to all species of *Heterodera* (Winslow, 1955*b*; Hesling, 1957).

TABLE 9. *The standard error for estimates of the logarithm of egg density with* Heterodera göttingiana *and* H. schachtii

Eelworm	Sample	Log. mean no. eggs/g. ± S.E.	Range of mean ± S.E. converted back to original units
H. göttingiana (Aldringham micro-plots)	*A*/1 *a*	2·116 ± 0·020	125–137
	A/1 *b*	2·094 ± 0·026	117–132
	A/2	2·129 ± 0·009	132–137
	A/3	2·141 ± 0·018	133–144
	A/4	2·141 ± 0·013	134–143
	A/5	2·110 ± 0·026	121–137
	A/6	2·140 ± 0·023	131–146
	A/7, 8	2·104 ± 0·011	124–130
H. schachtii	Isleham	1·680 ± 0·024	45·3–50·6
	Oxlode	1·383 ± 0·038	22·1–26·4
	Spalding	1·343 ± 0·044	19·9–24·4

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FIELD EXPERIMENTS ON CEREAL ROOT EELWORM

By L. E. W. STONE

*South-West Regional H.Q., N.A.A.S., Bristol**

In 1955–59 experiments were carried out in south-west England on field sites heavily infested by the cereal root eelworm, to determine the behaviour of the soil population under various crops.

Under three common types of ley, the population fell to less than 5 % of its original level in 3 years.

Of six pure grass stands tested, the population fell most sharply under cocksfoot and tall fescue.

Observation plots under the four cereals illustrate their different efficiencies as hosts.

In the south-west of England before 1950 the cereal root eelworm *Heterodera major* O. Schmidt, 1930 was merely of academic interest. Attacks, mainly on oats, became more frequent in the early 1950's, and by 1955 this eelworm was of considerable economic importance, particularly on the chalk downs of Wiltshire and Dorset (Stone, 1956). In the autumn of 1955 a series of field experiments was planned to determine the behaviour of the soil population under various cropping régimes. The use of the cheese-sampler type of tool enabled a large number of samples to be taken per plot (thus increasing the efficiency of the sample) without accumulating a large bulk of soil.

Laboratory methods

Soil from each plot from each sampling was air-dried, and passed through a 3 mm. sieve. The cysts were extracted by the usual technique (Fenwick, 1940). Possible cyst loss was checked by redrying the sediment from the Fenwick can and repeating the washing process. However, these experiments were sited on light, chalky soils, and few or no further cysts were recovered by this method. Furthermore, the soil samples were always kept dry for some time, and flotation of soil samples taken in the autumn was not carried out until the late winter or the following spring. Hesling (1956) has shown that there is a considerable loss of newly formed cysts when soil is washed in September, but Fidler, Church & Southey (1959) found very little loss of viable cysts from soil washed in the spring.

It was necessary to examine large numbers of cysts in these experiments. In practice, the cysts were soaked in water for 2–3 days, and examined in batches of five placed at equal intervals along microscope slides, each cyst in a drop of water. Each cyst was opened in turn; in the main, they were identified by larval length (Fenwick & Franklin, 1951), but morphological characters (Cooper, 1955) were also used where these were obvious. A dry finger was found to be a most efficient tool for removing cyst fragments and eggs of other lemon-shaped cysts. Cyst

* Now at South Wales subcentre, 66 Ty Glas Road, Cardiff.

fragments and eggs and larvae of *H. major* were washed into a measuring cylinder for aliquot estimations of the population, converted into eggs/g. of soil. Records were also kept of viable cysts, but for brevity the egg and larval counts only are presented here.

(1) *Effect of ley mixtures*

This experiment was sited on a field of shallow, chalky soil near Salisbury, Wiltshire, where oats had failed in 1954. Three types of ley mixture (given in Table 1) were sown on 12 September 1955, using a broadcast drill attached to a Cambridge roller. Each plot was 16 ft. wide (two drill widths), and extended the whole width of the field (approx. 300 yd.) for the convenience of the farmer.

TABLE 1. *Composition of ley mixtures*

Cockle Park type		Timothy–meadow fescue type		Rye-grass type	
lb./acre		lb./acre		lb./acre	
3	New Zealand Italian rye-grass	10	Danish meadow fescue	6	H.I. rye-grass
3	S. 24 perennial rye-grass	6	S. 48 timothy	6	S. 101 perennial rye-grass
3	S. 23 perennial rye-grass	3	Late-flowering red clover	3	Late-flowering red clover
5	S. 37 cocksfoot	1½	New Zealand permanent-pasture white clover	1½	New Zealand permanent-pasture white clover
2	Scandinavian timothy				
2	S. 48 timothy				
3	Late-flowering red clover				

Four replications were used, in a randomized block layout. The seed germinated well, but all plots eventually became somewhat clover-dominant. The plots were grazed in 1956, cut for hay in 1957, and grazed again in 1958. Throughout the experiment the plots retained their identity and were readily discernible. Soil samples were taken at sowing and subsequently in the spring and autumn of 1956, 1957 and 1958, but those taken in autumn 1957 were damaged in store and no results are available for this sampling. Samples were taken every ten paces walking parallel to the plot borders, about 4 ft. away from one border one way, and the same distance from the other border on the return journey. The sample from each plot was thus made up of approx. sixty individual borings, taken to the full depth of the soil (around 5 in.).

In Table 2 the populations at each sampling date are expressed as percentages of the original count (given in brackets). For statistical analysis the populations were transformed into logarithms. Six samplings, four blocks, and three leys give a total of 71 D.F. The only significant effect here was that due to seasons ($P < 0.001$); leys, blocks, and all interactions were non-significant. Table 3 presents the mean log. populations and their critical differences.

Points of note are:

(1) It would be expected that any appreciable new generation of cysts produced on the grass roots would be indicated in the autumn counts compared with those

of the preceding spring. In fact, in only one of these comparisons (Cockle Park, 1956) did any increase occur, and this was not significant (Table 3).

(2) The populations show a general decline, which was particularly marked during the winter of 1956 and from spring 1957 to spring 1958.

TABLE 2. *Effect of leys*

Percentage of original populations (in brackets) in eggs/g. soil. Means of four plots.

	Sampling date					
Ley	Autumn 1955	Spring 1956	Autumn 1956	Spring 1957	Spring 1958	Autumn 1958
Cockle Park	100 (16·5)	31·5	50·2	15·1	6·1	3·0
Tim/fescue	100 (17·2)	47·6	25·6	26·7	9·9	4·1
Rye-grass	100 (19·0)	43·9	24·5	22·8	8·2	4·4

TABLE 3. *Effect of leys*

Mean log. populations in eggs/g. soil.

	Sampling date					
Ley	Autumn 1955	Spring 1956	Autumn 1956	Spring 1957	Spring 1958	Autumn 1958
Cockle Park	1·23	0·77	0·87	0·50	0·29	0·16
Tim/fescue	1·24	0·92	0·72	0·72	0·41	0·21
Rye-grass	1·29	0·94	0·75	0·72	0·35	0·25
Overall	1·26	0·88	0·78	0·65	0·35	0·20

L.S.D. (individual means) 0·23 ($P = 0·05$), 0·31 ($P = 0·01$).
L.S.D. (overall means) 0·13 ($P = 0·05$), 0·18 ($P = 0·01$).

(3) Under all three leys the populations fell to less than 5 % of their original level in 3 years. It seems clear that, at any rate on chalky soil, the use of these types of ley mixture on eelworm-infested land is beneficial in reducing infestation as well as being good farming practice.

The mean dry weights of roots from each ley were estimated from samples taken in the autumn of 1958; they were: timothy–meadow fescue, 45·3 cwt./acre; rye-grass, 42·5; and Cockle Park, 37·2; with an L.S.D. ($P = 0·05$) of 7·4 cwt./acre. Where weight of root is desirable, timothy–meadow fescue may be the best ley to use.

(2) *Effect of pure grasses*

This experiment was sited on a portion of a field near Salisbury, Wiltshire, on which oats had failed due to cereal root eelworm in 1955. The grasses used and rates of sowing were as follows: S. 101 perennial rye-grass, 16 lb./acre; S. 22 Italian rye-grass, 25 lb./acre; S. 143 cocksfoot, 14 lb./acre; S. 215 meadow fescue, 14 lb./acre; S. 48 timothy, 10 lb./acre; and S. 170 tall fescue, 20 lb./acre. The seeds were sown by hand on plots 6 yd. square on 29 June 1956; there were four replicates, giving twenty-four plots in all. The plots were kept weed-free, fertilized, gapped-up where necessary, and cut by autoscythe when required each spring to simulate grazing.

Twenty soil samples were bulked from each plot before sowing and after 1, 2, and 3 years, and examined for cereal root eelworm. Table 4 presents the populations as percentages of the original count (given in brackets).

For statistical analysis, the data were transformed into logarithms. Four years, six grasses and four replications give a total of 95 D.F. In this experiment also the year effect was the most marked, but there was also a significant grass effect ($P < 0.01$) enabling comparisons to be made between grasses as well as years. None of the interactions was significant. Table 5 gives the mean log. populations and their critical differences.

TABLE 4. *Effect of pure grass species*

Percentage of original populations (in brackets) in eggs/g. soil. Means of four plots.

	Sampling date			
Grass species	Spring 1956	Spring 1957	Spring 1958	Spring 1959
Cocksfoot	100 (52·5)	20·5	19·5	4·5
Italian rye-grass	100 (71·3)	36·5	19·6	14·7
Perennial rye-grass	100 (57·0)	36·4	23·7	13·7
Timothy	100 (62·0)	39·1	29·8	13·8
Tall fescue	100 (46·0)	29·4	18·0	8·1
Meadow fescue	100 (48·5)	33·5	30·4	21·7

TABLE 5. *Effect of pure grass species*

Mean log. populations in eggs/g. soil.

	Sampling date			
Grass species	Spring 1956	Spring 1957	Spring 1958	Spring 1959
Cocksfoot	1·71	0·99	1·00	0·37
Italian rye-grass	1·84	1·39	1·15	0·93
Perennial rye-grass	1·74	1·29	1·10	0·79
Timothy	1·79	1·33	1·25	0·89
Tall fescue	1·66	1·05	0·88	0·48
Meadow fescue	1·68	1·20	1·15	1·00
Overall	1·73	1·21	1·09	0·74

L.S.D. (individual means) 0·27 ($P = 0.05$), 0·36 ($P = 0.01$).
L.S.D. (overall means) 0·11 ($P = 0.05$), 0·15 ($P = 0.01$).

Points of note are:

(1) There is again a general decline in population, particularly marked in the first year. The overall decline in 3 years is not so marked as under leys (Table 4, cf. Table 2) despite the higher initial populations.

(2) Although there is no significant difference between the initial populations, (Table 5), differences become apparent after the first year, and after 3 years the populations under cocksfoot and tall fescue are markedly less than those under the other grasses. Duggan (1958) found an increase in population in the second year under the rye-grasses in microplots. There is no evidence of that in this experiment,

although the decline in those populations at this stage is not significant (Table 5, lines 2 and 3, cols. 2 and 3).

Hesling (1958) has found that cocksfoot, perennial and Italian rye-grass are poor hosts of *H. major*, and that timothy is poorer still, when grown in pots for one season. In this experiment the populations behave in a very similar way under timothy and both rye-grasses throughout the 3 years, but under cocksfoot the decline is more marked (Tables 4, 5). It is evident that none of the grasses used here has produced many new cysts containing eggs.

In recovering the cysts from the final sampling of this experiment, it was noticed that there were marked differences in the amount of fibrous root present on the filter papers. This material was dried and weighed; the mean weights (per 200 g. soil sample) were as follows: cocksfoot 0·10 g., Italian rye-grass 0·18 g., perennial rye-grass 0·39 g., timothy 0·12 g., tall fescue 0·48 g., meadow fescue 0·30 g. Analysis gave a critical difference ($P = 0{\cdot}01$) of 0·09 g. It was felt that these data were worth recording, but the respective weights cannot take into account any fibrous root which had already decayed in the soil.

(3) *Effect of cereals*

Two-drill widths each of S. 147 winter oats, Capelle winter wheat, Pioneer winter barley and King II winter rye were sown in duplicate plots 50 yd. long on a portion of a field infested by *H. major* near Salisbury, Wiltshire, on 24 October 1956. The object was to demonstrate the effect of this eelworm on the four cereals, and to observe their effect on the soil population. Soil samples were taken from each plot before sowing and after harvest on 4 October 1956. Many cysts were observed on the oat roots during the summer, and the crop was pale and stunted; no obvious patchiness occurred in the wheat and barley, but a few cysts were found on the roots; no cysts were seen on the rye roots and the crop grew evenly and well. The data from the soil samples are summarized in Table 6.

TABLE 6. *Effect of cereals*

Populations in eggs/g. soil.

Plot	Initial population	Final population
Oats 1	120	93
Oats 2	58	113
Wheat 1	50	18
Wheat 2	91	48
Barley 1	57	45
Barley 2	82	64
Rye 1	92	7
Rye 2	104	15

Points of note are:

(1) Oats, as expected, was the best host. Although it failed to maintain an initial population of 120 eggs/g. (Oats 1, Table 6) it built up the population in the second plot from 58 to 113 eggs/g.

(2) In this trial, barley was a better host than wheat. In both plots, the population fell less under barley than wheat.

(3) Rye, as expected, was a very poor host. The populations fell sharply in both plots.

These observations confirm the work of Jones & Moriarty (1956), and Hesling (1959) on the order of host efficiency of the cereals, and general experience in the field.

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EXPERIMENTS ON THE AIRTIGHT STORAGE OF DAMP GRAIN

I. INTRODUCTION, EFFECT ON THE GRAIN AND THE INTERGRANULAR ATMOSPHERE

By MARY B. HYDE and T. A. OXLEY*

Pest Infestation Laboratory, Slough, Bucks

(With 7 Text-figures)

Airtight storage as a means of preventing deterioration of damp grain was studied both on a laboratory scale and in 10-ton bins, at grain moisture contents from 17 to 24 %.

Except when containers leaked, there was no development of mould, and the grain was bright and free-flowing, even after prolonged storage, at high moisture content. The grain remained mould-free after a bin was opened several times and small quantities of grain run out in cool weather. Such grain, removed from hermetic conditions and stored in sacks in an unheated building, showed no mould development for several weeks.

With prolonged hermetic storage or at high moisture content the grain developed a sour-sweet smell and taste which when extreme were not entirely removed by subsequent airing or drying.

There was no spontaneous heating; the grain in the 10-ton bins reflected the mean temperature of the surroundings.

The oxygen in the intergranular air was reduced to a low level and replaced by carbon dioxide within a few days or weeks, according to the moisture content and temperature of the grain. At moisture contents of 16 % and above, appreciable positive pressure developed inside the containers. The carbon dioxide concentration rose to 90–95 % at grain moistures of 22–24 %, 70–75 % at 19 %, 50 % at 18 %, and 35–40 % at about 17 % moisture content. The change from aerobic to anaerobic activity was marked by a reduction in the rate of production of carbon dioxide. With grain of less than 14 % moisture content, the rate of respiration was slow, the carbon dioxide concentration being only about 2 % after 18 months' storage. As long as oxygen remained, the apparent respiratory quotient was consistently between 0·6 and 0·7, whatever the moisture content of the grain.

Introduction

Storage in airtight containers seems to offer a possible means of preserving grain that is too damp to be kept safely under normal conditions. Interest in hermetic storage arose in France about a century ago to help solve the problems of provisioning garrisons and prisons and of feeding the 2000 horses of the General Omnibus Company of Paris (Müntz, 1881; Manessier, 1950). The method was later tested experimentally by Blanc (1938, 1939) on moderately damp grain (16·2 % moisture content).

* Present address: Tropical Products Institute, 56–62 Grays Inn Road, London, W.C. 1.

Resulting from the success of these tests, airtight storage has been developed commercially in France to store grain of moderately high moisture content, usually about 17%, but sometimes higher than this (Anon. 1955; Hyde, 1953; Pasfield, 1953). The bins used vary in capacity from 100 to 190 tons, assembled in groups of four to twenty-eight bins. They are constructed of welded steel, because brick, concrete, wood or riveted metal structures are not sufficiently gastight.

In tests in the United States with maize of high moisture content, up to 27%, Foster, Kaler & Whistler (1955) showed that in spite of some deterioration the grain could be fed satisfactorily to pigs.

Experiments by Teunisson (1954*a*, *b*) on rough rice at moisture contents up to 32%, in what were assumed to be airtight containers, showed that growth of certain micro-organisms, particularly mycelial yeasts, was still possible under the conditions of her tests. Evidence from the present work at the Pest Infestation Laboratory suggests, however, that Teunisson's containers were not completely airtight.

Investigation of the effect of airtight conditions on the chemical and other properties of stored grain have been carried out on a small scale by Peterson *et al.* (1956), Scriban (1949), and Shvetsova & Sosedov (1958).

The method has not yet been adopted commercially in either Great Britain or the United States. In an attempt to see if it could be used successfully in Great Britain for grain of high moisture content, thus delaying or eliminating the need for artificial drying, some experiments have been carried out at the Pest Infestation Laboratory, Slough. Very brief accounts during the progress of the work have been given by Oxley & Hyde (1955, 1957). The present paper gives a general account of the principle of the method, the experimental arrangements, and results relating to the general condition of the grain itself and the intergranular atmosphere. Changes of a more specific nature will be described in later papers in the series, when the general conclusions will also be discussed.

The principle of the method

As well as causing serious commercial damage themselves, insects or fungi present in grain stored under aerobic conditions can also initiate so-called 'heating' in the grain. If, however, infested or damp grain is kept in an airtight container the oxygen initially present will soon be used up and replaced by carbon dioxide, mainly due to the activity of the damaging organisms, for various authors (Bailey, 1940; Festa, Delidovich & Pyatenko, 1953; Hyde, 1954; Milner, Christensen & Geddes, 1947*a*; Milthorpe & Robertson, 1948) have shown that the respiration of the grain itself is low. When the oxygen concentration has fallen to a sufficiently low level, the respiration of the insects or moulds will be suppressed and they will die or become inactive before they have become numerous enough to cause serious deterioration. It was at first thought that accumulation of carbon dioxide was the lethal factor, but it is now established that depletion of oxygen is the primary cause of death or inactivity, although high concentrations of carbon dioxide can be harmful to both insects and moulds (Bailey, 1955, 1956, 1957; Barinova, 1953; Brown, 1922; Geddes, Cuendet & Christensen, 1955; Golding, 1940*a*, *b*, 1945;

Peterson *et al.* 1956). In the absence of oxygen, however, certain anaerobic organisms can continue to grow, and their development may result in damage to the grain.

Materials and methods

Scope of the tests

The main experiment, to determine whether the method, which had given promising results in a laboratory test, could be successful on a commercial scale in Great Britain, was carried out in three 10-ton steel bins, based on a French design. The bins were cylindrical, with conical tops, and mounted on legs so that the hopper bottoms could discharge directly into sacks. The welded sheet steel was 3 mm. (10 G) in thickness, painted externally with a red primer and then with an aluminium-bitumen paint. The lower slide openings and upper manholes were closed hermetically by plates bolted against sponge rubber gaskets. The bins were fitted at the top and bottom cones with needle valves to which were connected polythene air-sampling tubes. The lower of these were also connected to water manometers, enabling pressure changes to be measured and adjusted.

Because of the impracticability of taking grain samples from the sealed bins, smaller containers were used for controls, and also for small-scale tests at constant temperatures and with a more comprehensive range of moisture contents than was possible on a large scale. The smaller containers comprised preserving jars of 1100 and 1600 ml. (nominal 3 and 4 lb.) capacity, food cans of 500 and 900 ml. (1 and 2 lb.) capacity and narrow-necked 9600 ml. (2-gallon) cans. Test-tubes ($15 \times 1{\cdot}9$ cm.) were also used for tests on viability.

The jars were fitted with rubber gaskets and metal closures, and the 2-gallon cans with sealed-in rubber bungs, through which suitably closed tubes were inserted, so that samples of the intergranular air could be withdrawn for analysis at intervals during the test. The smaller cans were sealed by machine in the normal way, and each was sampled once only, the flat metal end being pierced by a special device which allowed a gas sample to be taken without entry of air.

Observations made

When a container was filled or opened the grain was examined for general appearance. The colour of the grain was noted, and whether or not it showed signs of mould visible to the naked eye or through a $\times 10$ lens. Its state, whether free-flowing or caked and compacted, was observed, and its moisture content, viability and microflora determined. The smell of the intergranular air, above the grain mass or through the air-sampling tubes, and the smell and taste of the grain itself were assessed, if possible by several observers. Certain chemical properties and milling and baking properties were also studied. Some tests were carried out on the effect of feeding hermetically stored grain to poultry and insects.

Observations were made at intervals during storage on the intergranular concentrations of oxygen and carbon dioxide, on the temperature changes in the bulk and on pressure changes in the bins.

The following methods were used for the properties described in the present paper.

Moisture-content determination

The moisture content of the grain was established by thorough sampling and measurement. Normally it was determined on 5–10 g. samples ground on a Regent mill (no. 2 setting) and dried in a ventilated oven at 113° C. for 4 hr. Because of difficulties in grinding the dampest grain, its moisture content was determined by a two-stage method. The results were expressed on a wet-weight basis.

Temperature measurement

Temperatures in the 10-ton bins were indicated by copper-constantan thermocouples, either recording directly on a multi-point recorder or read daily by means of a potentiometer. Initially three thermocouples were installed in each bin, one at the centre of the grain mass and one (external) on both the north and south faces. After the first season only the temperature at the centre of each bin was recorded, as observations had shown that the temperatures at the bin surfaces reflected changes due to the weather more conveniently recorded elsewhere.

Thermohygrograph records were maintained out-of doors in a Stevenson screen and in a louvred box near the silo bins. Laboratory thermohygrographs recorded the temperatures in the constant-temperature rooms used for many of the small-scale tests. Temperatures in the other rooms were not recorded continuously, but were noted from time to time.

Gas analysis

The concentrations of carbon dioxide and oxygen were determined gasometrically in samples of the intergranular air, taken into hypodermic syringes, lubricated with medicinal paraffin, after suitable flushing of the connecting tubes. Either a standard Haldane apparatus was used, or one of two modifications of it designed to take smaller samples (10 ml.) and graduated for a greater proportion of the volume. One of these was graduated for 97 % of the volume, but measured carbon dioxide only; the oxygen concentration, in the bins only, was then determined on a second air sample drawn through a bubbler containing a concentrated solution of potassium hydroxide, to absorb the carbon dioxide and bring the measurements on the residual gas within the limits of the standard apparatus.

Absorption of carbon dioxide was in a 40 % (w/v) solution of potassium hydroxide, and of oxygen in a form of Fieser's solution, i.e. an alkaline solution of sodium dithionite with sodium anthraquinone disulphonate as accelerator.

Pressure changes

The pressures in the 10-ton bins were read daily at 9.0 a.m. on water manometers attached by three-way taps to the lower air-sampling tubes.

Grain used

Apart from some preliminary tests with Yeoman wheat and an unnamed variety of barley, most of the experiments were carried out on Atle wheat, although later Kenia barley was included. Generally, the high moisture content required was obtained by harvesting locally grown grain by combine harvester somewhat prematurely, while the grain was still drying out on a drying curve the form of which had been established from previous observations at the laboratory over a number of seasons. The hazards of the weather at harvest-time, however, made it difficult to obtain the exact moisture content required. Some of the laboratory-scale experiments were carried out on specially purchased high quality seed wheat, re-wetted and artificially dried down to give a more complete moisture range than was otherwise possible.

In the first season of large-scale tests the grain was used directly as it came from the combine harvester, but in later years it was passed through a simple winnowing and cleaning machine before use, thus removing much green material, chaff and unthreshed ears, foreign weed seeds, and various field insects. As the grain was being fed into the elevator to the bins, a small quantity was taken from each sack and these samples were mixed to form a 'bulk' sample from which the small-scale tests were set up, and from which samples for germination tests, chemical analysis and milling and baking tests were taken. Samples for determination of moisture content were taken at frequent intervals during the filling of the bins, and also from the 'bulk' sample.

Summary of the experimental conditions

A summary of the various conditions under which the experiment has been carried out is given in Table 1. For most types of grain two types of control were used: (*a*) grain dried at harvest and kept (i) under airtight and (ii) under non-airtight conditions, and (*b*) grain stored damp under non-airtight conditions.

The reference numbers in Table 1 are given in the text and later tables in square brackets, where appropriate, to identify the grain concerned.

Results and discussion

Appearance, smell and taste of the grain

The results of visual examination of the grain when the various types of container were opened are given in Tables 2 and 3. Whenever the container had remained airtight the grain was without exception free from visible mould and generally free-flowing, even at high moisture contents (up to 23 %) and for prolonged periods of storage (81 weeks or more). At the higher moisture levels or with extended periods of storage there was sometimes, in the lower part of the container, a certain amount of compaction due to pressure on the relatively soft grains by the grain above. This temporary 'caking' was easily dispersed and was quite distinguishable from true caking due to mould growth.

TABLE 1. *Summary of the experiments*

Ref. no.	Grain		Moisture (%)	Temp. of storage (°C.)	Container	Remarks
1*c*	Barley	1952	21·0 14·7	Variable (room)	Cans (1 lb.)	Unknown origin
2*b*	Wheat (Yeoman)	1952	19·1 12·4	20	Jars (4 lb.)	Grown at laboratory and hand-harvested
3*a*	Wheat (Atle)	1953	23·2	Variable (recorded)	Bins (10 ton)	—
3*b*	—	—	23·3 23·3* 13·8	15	Jars (4 lb.)	*CO_2 added before sealing
3*c*	—	—	23·3 14·6	Variable (outdoor)	Cans (1 lb.)	—
4*a* (i)	Wheat (Atle)	1954	22·7	Variable (recorded)	Bins (10 ton)	—
(ii)			18·9*			*CO_2 added before sealing
4*b*	—	—	22·7 18·9 13·5	15	Jars (4 lb.)	—
4*c* (i)	—	—	22·7 18·9 18·9* 13·5	Variable (unheated room)	Cans (1, 2 lb.)	Sealed immediately *CO_2 added before sealing
(ii)	—	—	23·2		Cans (1, 2 lb.)	Allowed to go mouldy before sealing
5*c*	Wheat (Atle)	1954	22·0	25	Cans (1 lb.)	Conditioned from dry state before canning
5*d*	—	—	Range, 17·9–24·4	15–18	Cans (2 gal.)	Conditioned from dry state and re-dried before canning
5*e*	—	—	Range, 16·5–22·4	18	Test-tubes	Tested for germination only
6*a*	Wheat (Atle)	1955	17·0	Variable (recorded)	Bins (10 ton)	—
6*b*	—	—	17·0 14·0	15–18	Jars (4 lb.)	—
6*c* (i)	—	—	26·3	15–18	Cans (1 lb.)	Tested for germination and microbiology only
(ii)	—	—	17·0 14·1	15–18	Cans (1, 2 lb.)	—
6*e* (i)	—	—	Range, 17·6–20·7	15–18	Test-tubes	Tested for germination only
(ii)	—	—	Range, 17·4–20·6	25	—	—
(iii)	—	—	Range, 12·6–22·5	15	—	—
(iv)	—	—	Range, 12·6–22·5	25	—	—
7*a* (i)	Barley (Kenia)	1955	19·2 16·7	Variable (recorded)	Bins (10 ton)	—
(ii)						
7*b*	—	—	19·2 16·7 12·1 9·9	15–18	Jars (4 lb.)	—
7*c*	—	—	19·2 16·7 12·1	15–18	Cans (1, 2 lb.)	—

TABLE 2. *Condition of grain on opening container—Wheat*

Storage (weeks)	Moisture content (%)	State	Colour	Degree of mouldiness	Smell	Taste
			Yeoman wheat (1952 crop), jars (20° C.) [2 *b*]			
13*	12·7	Free-flowing	Normal	None	Normal	Normal
52†	12·5	Very free-flowing	Normal	None	Normal	Normal
7‡	19·1	Slightly caked at top	Normal	Some, at top	Sour-sweet	Sour
13	19·2	Compacted in lower third; free-flowing at top	Bright	None	Sour-sweet	Sour
26	19·5		Bright	None	Sour-sweet	Sour
27§	19·1	Caked	Dull	Considerable	Musty	Unpleasant
52	19·1	Free-flowing at top; compacted lower down	Bright	None	Sour-sweet	Sour
			Atle wheat (1953 crop), bins (variable temperature) [3 *a*]			
15	25·2	Free-flowing	Bright	None	Strong sour-sweet	Sour
33	24·0	Compacted at base of bin; free-flowing above	Bright	None	Strong sour-sweet	Bitter
81	24·5		Bright	None	Strong sour-sweet	Sour
			Atle wheat (1953 crop), jars (15° C.) [3 *b*]			
13*	13·9	Free-flowing	Normal	None	Not abnormal	Normal
13	23·9	Slightly compacted	Very bright	None	Strong sour-sweet	Sour
23	24·1	Slightly compacted	Bright	None	Fruity	—
23‖	23·7	Normal	Bright	None	Slightly fruity	—
			Atle wheat (1953 crop), cans (variable temperature) [3 *c*]			
6†	14·0	Free-flowing	Normal	None	Normal	Normal
24†	14·1	Free-flowing	Normal	None	Normal	Normal
117†	13·8	Free-flowing	Normal	None	Normal	Normal
3	23·8	Free-flowing	Bright	None	Sour-sweet	Sour
6	24·8	Very free-flowing	Bright yellow	None	Sour	Sour
24	24·2	Free-flowing	Bright yellow	None	Sour-sweet	Sour
117‡	24·2	Mostly free-flowing	Mostly normal	Slight	Tobacco-like	—
			Atle wheat (1954 crop), bins (variable temperature) [4 *a*]			
31·5‖	18·8	Free-flowing	Normal	None	Very slightly sour	Slight taint
39‖	19·1	Free-flowing	Normal	None	Slightly sour	—
11	23·8	Compacted below; free-flowing above	Bright	None	Sour-sweet (less than in 1953)	Sour
18	23·4	Free-flowing	Bright	None	Sour-sweet	—
31·5	22·8	Free-flowing	Normal	None	Sour-sweet	—
36	23·2	Free-flowing	Normal	None	Sour-sweet	—
			Atle wheat (1954 crop), jars (15° C.) [4 *b*]			
71	19·0	Free-flowing	Normal	None	Sweet-sour	—
115	19·2	Slightly compacted	Slightly orange	None	Sour-sweet	—
60	22·7	Free-flowing	Normal	None	Strong sour-sweet	—
115	23·0	Compacted; some soft grains	Bright; slightly orange	None	Strong sour-sweet	—
			Atle wheat (1954 crop), cans (variable temperature) [4 *c*]			
34†	13·1	Free-flowing	Normal	None	Normal	Normal
63†	13·4	Free-flowing	Normal	None	Tobacco-like	—
9	18·6	Free-flowing	Normal	None	Hardly abnormal	Nearly normal
19	18·9	Free-flowing	Normal	None	Slightly honey-like	Slightly sweet
19‖	18·8	Free-flowing	Normal	None	Similar, but slighter	Slight
34‡	18·4	Free-flowing	Normal	Some	Mushroom-like	Slightly abnormal
60	18·7	Free-flowing	Normal	None	Hardly abnormal	—
62	18·6	Free-flowing	Normal	None	Slightly abnormal	Sour
71‡	18·7	Some caking	Discoloured	White patches of mould throughout	Slightly musty	—
19	23·5	Free-flowing	Normal	None	Sour-sweet	—
34‡	23·2	Partly caked	Discoloured	Visible throughout	Yeast-like	Abnormal, not strong
62	23·1	Free-flowing	Normal	None	Sour-sweet	—
62‡	23·4	Some caking	Discoloured	Mouldy	Yeast-like	—
			Atle wheat (1955 crop), jars (15° C.) [6 *b*]			
14	17·1	Free-flowing	Normal	None	Normal	No taint
74	17·6	Normal	Normal	None	Very sweet	—
74	17·7	Normal	Normal	None	Very sweet	—
			Atle wheat (1955 crop), cans (15° C.) [6 *c*]			
63	16·9	Normal	Normal	None	Very sweet	—

* Non-hermetic; dried at harvest. † Hermetic; dried at harvest. ‡ Faulty seal; not completely airtight. § Non-hermetic; undried. ‖ CO_2 added before sealing.

In most instances there was no change in colour during hermetic storage, especially at the lower moisture levels. There was a tendency at the higher moisture contents for the colour to become brighter than that of the dried control grain. This was particularly noticeable in the barley sealed in small aluminium cans in 1952 [1*c*], some of which assumed a quite bright yellow colour, but it was also observed in wheat of all seasons. There were one or two instances, in all types of container at the higher moistures, of a few of the grains becoming swollen, soft and brown. It is possible that these grains were those which were still immature at harvest.

TABLE 3. *Condition of grain on opening container—Barley*

Storage (weeks)	Moisture content (%)	State	Colour	Degree of mouldiness	Smell	Taste
			Barley (1952 crop, cans (variable temperature) [1*c*]			
15	14·8	Free-flowing	Normal	None	Normal	Not unusual
25	14·7	Free-flowing	Light yellow	None	Normal	—
37	14·7	Free-flowing	Normal	None	Normal	—
12	19·0	Free-flowing	Bright yellow	None	Slightly sour	Slightly sour
18	21·0	Free-flowing	Normal	None	Sour	—
42	21·0	Free-flowing	Normal	None	Sour-sweet	—
22*	22·5	Free-flowing	Very bright yellow	Very slight on some grains	Slightly musty	—
42*	23·1	Free-flowing	Dull	White, on some grains	Sour-sweet	—
			Kenia barley (1955 crop), bin (variable temperature) [7*a*]			
38	16·7	Free-flowing	Normal	None	Barely detectable sour-sweet	Very slight taint
			Kenia barley (1955 crop), cans (15° C.) [7*c*]			
14	16·9	Free-flowing	Normal	None	No noticeable taint	No taint
39*	16·9	Some caking	Discoloured	Some small mould patches	Musty cheese	Taint
63	16·7	Free-flowing	Normal	None	Sour-sweet	—
16	19·2	Free-flowing	Normal	None	Slightly sweet	—
21	19·5	Free-flowing	Normal	None	Of carbon dioxide	—
31	19·6	Free-flowing	Normal	None	Sour-sweet; fruity	—
60	19·4	Free-flowing	Normal	None	Very sour; sweet later	—

* Faulty seal; not completely airtight.

As mentioned above, on all occasions where the container remained airtight, there was no sign of visible mould growth on the grain. A few containers, however, mostly small cans, although apparently sound, had imperfect seals, usually indicated by lack of 'blowing' of the can, little or no positive pressure inside when sampled, and a lower than expected concentration of carbon dioxide. In a number of these containers visible mould had developed, either in a few isolated patches or generally throughout the can, or there was a musty or yeast-like smell indicating growth of micro-organisms. Although at first regarded as a measure of failure, these cans later came to be considered a useful indication that moulds or other micro-organisms can grow if there is even only a slight leak, and emphasized the necessity for absolute airtightness to prevent mould growth on damp grain.

At the higher moisture levels, above about 16–17 %, the intergranular air and the grain itself developed a characteristic smell which can best be described as 'sour-sweet', and which was obviously alcoholic in nature. If the concentration of carbon dioxide was high, the air, particularly in the 10-ton bins, frequently had a pronounced smell of 'soda-water' due to the presence of the carbon dioxide.

The intensity of the smell increased with length of storage, moisture content and temperature. In general (see Tables 2 and 3), the smell was more sweet than sour at the lower moistures. If not too pronounced, the smell could be more or less entirely removed by airing, for example, in barley of 16·7 % moisture content [7*a*(ii)] which had been stored in a 10-ton bin for 38 weeks. At higher moistures or with more prolonged storage, however, the smell was not removed by drying or airing and was even carried over to the flour and to a lesser extent to the bread made from it.

Associated with the smell, the grain had a sour-sweet or bitter taste which again was more pronounced at higher moistures, or with longer storage, when it persisted even after airing or drying the grain, and in extreme cases was detectable in the bread.

Behaviour of damp grain after removal from hermetic conditions

Although the grain was normally mould-free when removed from airtight storage, it was not known how long it would remain so, without drying, when exposed to ordinary atmospheric conditions, a point of considerable practical importance.

To study this, thirty hundredweight of wheat of 23·2 % moisture content [3*a*] were run, when the bin was first opened after 35 weeks' hermetic storage, into thirty autoclaved sacks. These were built into an approximately cubical stack in an unheated wooden building, whose concrete floor was covered with two layers of bituminous roofing felt. Temperatures were recorded by thermocouples at various positions in the stack.

There was (Fig. 1, Stack I) no appreciable rise in temperature for about 6 weeks, during which time the ambient and stack temperatures varied about the initial mean temperature of approximately 9° C. After this time the grain began to heat rapidly, a change apparently initiated by a brief spell of warm weather. Patches of mould then began to develop on the sacks. Many of these patches were later found to extend to several centimetres into the grain; most seemed to consist of a single species of mould. *Aspergillus candidus* and *A. glaucus* occurred most commonly. Other fungi present included *Cladosporium herbarum*, *Penicillium* spp., *Syncephalastrum* sp. and *Botrytis cinerea*, as well as some unidentified forms.

On breaking down the stack after 3 months' storage mould patches were found to be distributed throughout the sacks. This suggests that they arose from surviving micro-organisms, or chance contaminants picked up as the grain was being run into the sterile sacks, rather than by invasion of the stack from outside. The relatively small number of mould patches that developed suggests that these survivors, or contaminants, were few in number.

Similar results were obtained with wheat of the next season's harvest, which ha

been hermetically stored at 22·7% moisture content [4*a*(ii)]. A stack as before was constructed when the bin was opened for the first time after 11 weeks' storage. Ambient and stack temperatures (Fig. 1, Stack II) were at first similar to those in the previous experiment but fell gradually with the advance of winter. After about 30 days, however, the temperature at several positions in the stack began to rise appreciably and 3 weeks later it became evident that this was due to invasion of the stack by mice. The stack was immediately fumigated with a mixture of carbon tetrachloride and ethylene dichloride, and all rise in temperature ceased. The possibility that the fumigation may have affected the development of fungi cannot

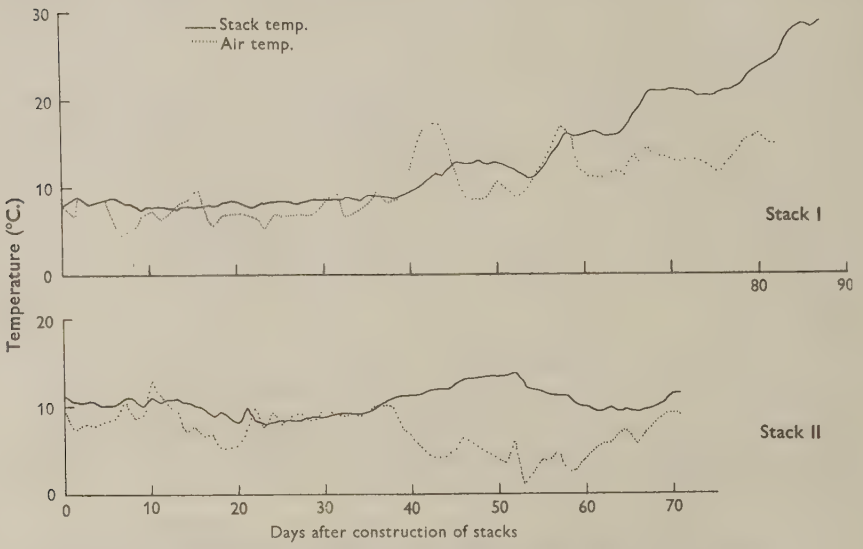

Fig. 1. Temperatures in stacks of hermetically stored grain after removal from airtight conditions.

be excluded, although these fumigants are not particularly toxic to moulds. Thus although the stack was found to be only very slightly mouldy when broken down 74 days after construction, it is possible that the condition might have been worse after that time if fumigation had not been necessary. On the other hand, the very low air temperatures then prevailing, averaging about 5° C., were too low for appreciable growth of moulds.

The results of these two tests seem to show that hermetically stored grain can be kept undried and in a mould-free state for some weeks after removal from hermetic conditions, provided that the air temperature is reasonably low.

Effect on the grain of repeated opening of a container

In practice, a user might not wish to empty an airtight bin all at once, but might prefer to remove small quantities of grain at intervals.

In an attempt to assess the effect on the grain of opening the container several times, a 10-ton bin filled with Atle wheat of 22·7 % moisture content [4*a*(i)] was opened four times at intervals of a few weeks during the winter and spring of 1954–55, and a small amount of grain was run out each time. The condition of the grain showed no further deterioration, on subsequent openings, from that observed when the bin was first opened after 11 weeks' storage (see Table 2). Although aerobic organisms were still active and soon used up the small amount of oxygen introduced each time the bin was opened, they were not present in sufficient quantity to produce visible mould growth. On each occasion the grain was free-flowing and mould-free, but had the typical sour-sweet smell and taste of hermetically stored damp grain.

In the above test, only about a ton of grain was removed each time the bin was opened, so that the bin was still more than half full after the four openings in cool weather. When this bin was later emptied completely, after 100 weeks' storage, by removing half a ton of grain every day over a period of 3 weeks during warm weather, there was noticeable deterioration of the grain. Although free from mould at first, small patches of mouldy, caked grain developed during the period taken to empty the bin.

Temperature changes in the bins

The 10-ton bins were the only containers used which were large enough to have shown spontaneous heating had any occurred. At no time were any temperatures recorded high enough to give any suggestion of 'heating' of the grain.

During the whole of the first season and for the first 2 months of the 1954–55 season, i.e. for the first 67 weeks of the large-scale experiment, the bins stood in the open air without any cover. The bins were not uniformly exposed to the sun and wind, but they protected or shaded each other to varying extents, which may explain the differences in temperature between the individual bins during this period. In November 1954 a Dutch barn was built over the whole silo installation, screening the bins from direct sunlight and strong winds, and also providing slight protection from radiation frost. This may account for the closer agreement between the bins and for the smoother temperature curves during the subsequent seasons.

As expected, the temperatures at the walls of the bins, measured during the first season only, fluctuated with the temperature of the outside air, and changed considerably during the course of a day. The temperatures at the centres of the bins, on the other hand, were not affected by diurnal changes in air temperature and did not vary noticeably during a 24 hr. period, but showed only seasonal fluctuations. The temperatures (weekly averages) at the centres of the bins for the first 4 years of the large-scale experiment are given in Fig. 2. Also plotted on this figure are the mean temperatures for each month (i.e. the mean of the monthly

mean maximum and minimum temperatures) at London Airport,* taken from the Meteorological Office's *Monthly Weather Reports* (Air Ministry, 1953–57).

Several interesting points emerge from these results:

(i) The temperature of the grain at the time of harvest was always considerably higher than the corresponding mean air temperature, but there was never any rise to temperatures which could be regarded as commercial heating.

(ii) The grain cooled progressively during the autumn following harvest, and thereafter the grain temperature reflected the mean temperature of the air to a remarkably close degree, rising in the spring and falling in the autumn.

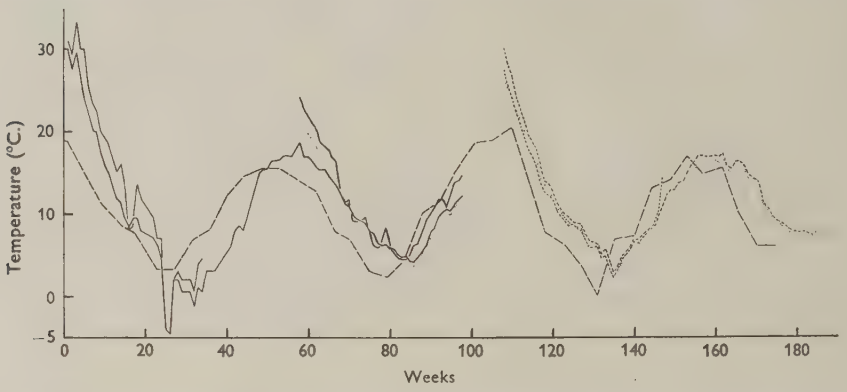

Fig. 2. Temperatures at the centres of 10-ton bins containing grain under airtight conditions. —— at 23% moisture content; .... at 19%; ---- at 17%. ——— Temperature at London Airport.

(iii) In all seasons studied there was a time lag between air-temperature changes and the corresponding changes in temperature at the centres of the bins. Assuming that the air temperature at London Airport closely represents that of the air surrounding the bins, the lag can be estimated approximately from the curves in Fig. 2. It appears to vary between 6 and 8 weeks.

It is interesting to compare this lag with that which would be expected to occur, on theoretical grounds, between the surface and centre temperatures of bins of this size. A close correspondence is not to be expected, however, since the surface temperature is affected by radiation, as well as by air temperature, and does not follow precisely the sine wave which is assumed by the theory. The lag calculated according to the method proposed by Babbitt (1945), and using the value found by him for the thermal diffusivity of wheat, is 8·9 weeks. In the circumstances this may be regarded as reasonably close agreement with the observed value.

(iv) When the grain was retained in the bins for more than one season (one bin in 1953–55 and two in 1955–57), the temperature at the bin centre did not rise during the second summer to the high figure at harvest, nor to the temperature of the newly harvested grain of that season, but the maximum was very close to

* London Airport is 5 miles (8 km.) E.S.E. from the site of the bins.

the maximum mean air temperature. In 1954 the temperature in the one bin remaining filled during the summer rose a little higher than the maximum mean air temperatures. This was before the construction of the Dutch barn and the bin was exposed to the summer sun, whereas the mean air temperatures are those in a Stevenson screen at London Airport. In the 1956 season the bins were protected from the sun by the barn, and the temperatures at their centres agree very well, with the 6–8 week time lag already mentioned, with the mean air temperatures.

(v) Except in the extremely cold spring of 1954, when the grain temperature fell to $-4{\cdot}5^{\circ}$ C., the minimum temperature reached by the grain was never quite as low as the minimum mean air temperature. In 1954 the bins were still in the open, not protected by the Dutch barn. The spring of 1956 was again exceptionally cold for a short period and the grain temperature fell sharply a few weeks after the cold spell. The very mild winter and spring of 1956–57 are reflected in the much higher minimum temperatures of the grain during that season.

Changes in the composition of the intergranular atmosphere

The concentrations of carbon dioxide and of oxygen (when determined) in the intergranular air are given in summarized form in Table 4 for the 10-ton bins and preserving jars, in Fig. 3 for a series of tests using 2-gallon cans, and in Fig. 4 for one season in the bins, in relation to length of storage. The values, for carbon dioxide only, for the smaller cans were too variable to allow a numerical tabulation to be drawn up, but they reflect the general trend shown in the other types of container.

Carbon dioxide concentration

The amount of carbon dioxide rose very rapidly during the first few days or weeks of storage, and then more gradually over the next few weeks or months, to reach a more or less constant value. Taking the containers as a whole, this maximum value was 90–95 % for grain moisture contents of 22–24 %, 70–75 % for moisture contents of 19 %, about 50 % for moisture contents near 18 %, and 35–40 % for moisture contents of about 17 %. With dry grain, 14 % moisture content, the carbon dioxide concentration was only about 2 % after more than 18 months' storage.

In most types of container there was generally a very slow progressive decrease in concentration of carbon dioxide after the maximum had been maintained for some time. It is assumed that this fall may have been due to very slow diffusion of the gas outwards through the gasket or other form of seal, or possibly to absorption of carbon dioxide by the grain itself.

At grain moisture contents of 16 % and above, the amount of carbon dioxide produced was more than the oxygen originally present indicating the occurrence of anaerobic respiration. The change from aerobic to anaerobic activity was characterized by a slowing up in the rate of carbon dioxide production, illustrated by the change of slope in the curves in Figs. 3*a* and 4*a*, which always took place when the carbon dioxide concentration was about 15–18 %, at which time the oxygen first reached its lowest level.

TABLE 4. *Intergranular concentration of carbon dioxide and oxygen*

Grain ...	Yeoman wheat	Atle wheat				Atle wheat						Kenia barley						Atle wheat		
Year ...	1952	1953				1954						1955						1955		
Container ...	4 lb. jars	10-ton bin		10-ton bin	4 lb. jars	10-ton bin *		10-ton bin		4 lb. jars	4 lb. jars	4 lb. jars	10-ton bin		10-ton bin		4 lb. jars	4 lb. jars	10-ton bin		4 lb. jars
M/C (%) ...	19·1	23·2		23·2	23·2	18·9		22·7		13·5	18·9	22·7	16·7		19·2		16·7	19·2	17·0		17·0
Temp. (° C.) ...	20	Variable		Variable	15	Variable		Variable		15	15	15	Variable		Variable		15	15	Variable		15
Ref. no. ...	[2 *b*]	[3 *a*]		[3 *a*]	[3 *b*]	[4 *a* (ii)]		[4 *a* (i)]		[4 *b*]	[4 *b*]	[4 *b*]	[7 *a* (ii)]		[7 *a* (i)]		[7 *b*]	[7 *b*]	[6 *a*]		[6 *b*]
	CO_2 (%)	CO_2 (%)	O_2 (%)	CO_2 (%)	CO_2 (%)	CO_2 (%)	O_2 (%)	CO_2 (%)	O_2 (%)	CO_2 (%)	CO_2 (%)	CO_2 (%)	CO_2 (%)	O_2 (%)	CO_2 (%)	O_2 (%)	CO_2 (%)	CO_2 (%)	CO_2 (%)	O_2 (%)	CO_2 (%)
Days																					
0	—	—	—	—	—	—	—	—	—	—	—	—	2†	18†	—	—	—	—	0·8	20	—
1	—	—	—	—	—	97	—	—	—	—	—	—	6	12	10	6	—	—	3	16	0·4
2	—	41	—	44	—	—	—	12	2·2	—	—	—	11	0·6	18	0·1	1	7	6	12	—
5	—	57	—	—	—	—	—	19	0·8	—	—	20	17	0·1	25	0·1	—	15	14	1	—
9	—	84	—	84	—	—	—	30	0·8	—	13	25	20	0·1	38	—	4	—	16	0·1	3
Weeks																					
2	—	90	—	90	—	—	—	48	—	—	12	—	23	·3	47	—	6	21	19	0·4	7
3	—	96	—	96	60	81	—	61	—	—	15	—	25	0·2	57	—	9	27	21	0·2	10
4	—	—	—	—	—	—	—	70	—	—	16	—	27	—	63	—	11	31	23	—	11
5	33	—	—	—	—	76	0·1	75	0·2	—	—	70	29	—	67	—	13	36	—	—	12
7	42	—	—	—	—	72	—	82	0·1	—	17	75	31	—	71	—	14	40	25	0·4	—
9	—	—	—	—	—	65	—	85	0·1	0·3	23‡	80	33	—	77	—	—	49	—	—	14
11	—	—	—	—	87	61	—	84	0·1	—	—	82	33	—	78	—	—	53	25	—	—
13	57	94	—	95	88	60	0·1	75	4·5	—	28	87	—	—	—	—	15	57	—	—	14
15	—	95	—	72	—	—	—	74	0·1	—	30	87	33	—	79	—	—	58	—	—	—
18	—	95	—	86	—	55	—	75	0·1	0·8	34	92	33	—	—	—	16	63	25	0·6	17
21	—	94	—	84	—	—	—	69	4·6	—	37	91	33	—	74	—	18	65	—	—	17
25	56	94	—	80	96	50	—	66	0·2	—	35	91	—	—	76	—	—	70	23	—	—
30	—	96	—	81	—	51	0·2	59	2·6	—	39	92	—	—	—	—	—	76	—	—	—
35	—	—	—	—	—	51	0·2	65	0·2	—	45	—	36	—	78	—	—	—	25	—	—
40	60	79	—	—	—	—	—	59	0·3	—	45	91	40	0·3	—	—	—	—	—	—	—
50	62	—	—	—	—	—	—	—	—	—	51	—	—	—	83	—	—	—	35	0·3	—
70	—	79	0·05	—	80	—	—	—	—	—	50	88	—	—	60	0·5	—	—	32	0·6	35
80	—	76	0·2	—	—	—	—	—	—	2·0	50	90	—	—	53	2·5	33‡	—	—	—	—
90	—	19	1·0	—	—	—	—	—	—	—	—	—	—	—	46	2·0	—	—	31	0·8	—
120	48	—	—	—	—	—	—	—	—	—	48	85	—	—	33	0·2	—	—	32	0·1	—
180	—	—	—	—	75–86	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

A single horizontal line indicates the container was opened. A double horizontal line indicates the container was emptied.
* CO_2 gas added before sealing. † At bottom of bin. ‡ O_2, 0·5 %.

The general rate of increase in carbon dioxide varied not only with the moisture content of the grain but also with the temperature. For instance, in preserving jars kept at 15° C. the rate of carbon dioxide production in all seasons was noticeably slower than in silo bins containing comparable grain, where the temperature in the

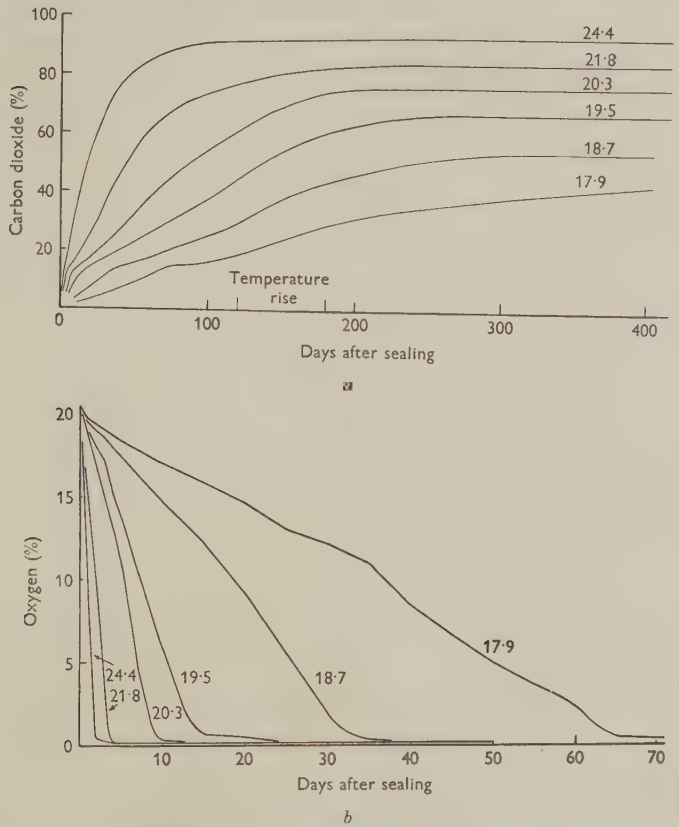

Fig. 3. Concentration of carbon dioxide (Fig. 3*a*) and of oxygen (Fig. 3*b*) in the intergranular atmosphere in 2-gallon cans, containing wheat at the percentage moisture contents indicated.

first few weeks of storage, when activity was greatest, was considerably higher than 15° C. The maximum values eventually reached were not, however, appreciably different in the two types of container (see Table 4).

The concentration of carbon dioxide finally reached therefore appears to be related more directly to the moisture content of the grain than to the temperature experienced, and probably represents the value at which the respiratory activity of the anaerobes characteristic of that particular moisture content ceases.

Oxygen concentration

Various difficulties prevented measurement of oxygen in some of the containers, but the results that are available enable a general picture to be drawn. The oxygen was consumed very rapidly indeed in the silo bins, reaching a value of 0·1–0·2 % in a few days or weeks according to the grain moisture content and the season (Fig. 4*b*). It then remained generally at a very low level for the rest of the storage period, as long as the containers remained unopened.

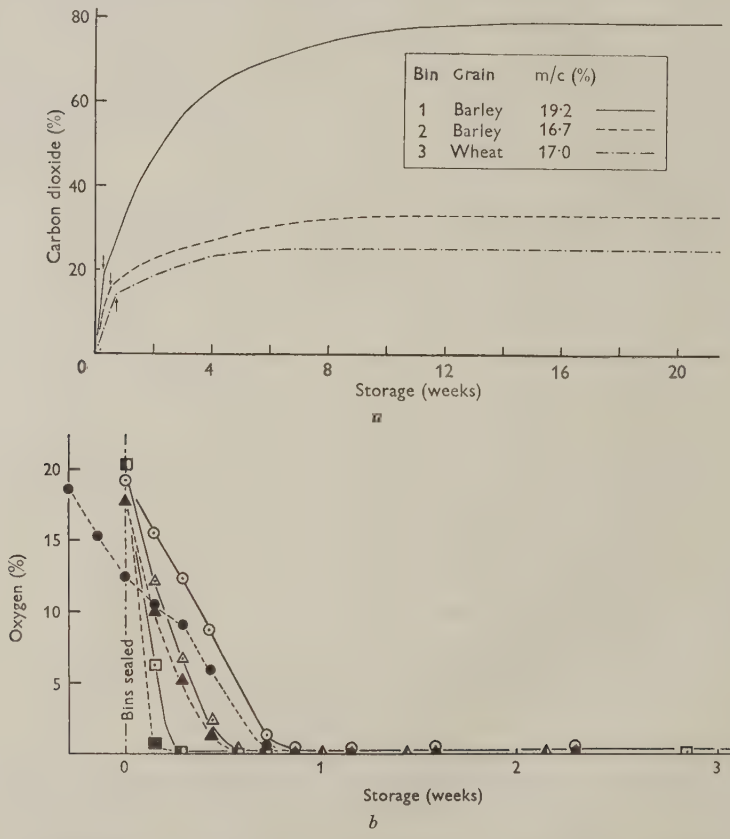

Fig. 4. Intergranular concentration of carbon dioxide (Fig. 4*a*) and of oxygen (4*b*) in 10-ton bins containing grain under hermetic conditions. The arrows indicate the times by which all the oxygen had been consumed.

Grain	Moisture (%)	Position in bin: Top	Position in bin: Bottom
Barley	19·2	⊡——	■- - -
Barley	16·7	△——	▲- - -
Wheat	17·0	⊙——	●- - -

The relation between fall in oxygen and grain moisture content is seen particularly clearly in the test using 2-gallon cans at 15° C. (Fig. 3*b*); at 24·4 % moisture content the oxygen was reduced to the minimum value in 2 days, whereas at 17·9 % moisture content it was 70 days before all the oxygen had been consumed, with corresponding intermediate periods for intermediate moisture contents. There is thus a definite connexion between moisture content and rate of consumption of oxygen for any one temperature.

In none of the types of container used did the oxygen concentration fall appreciably below 0·1 %, a figure well above the blank for the apparatus. This constant low value apparently represented the concentration below which there was no further aerobic respiration of the grain itself or of the micro-organisms associated with it, although anaerobic respiration was taking place.

The results for the bins given in Table 4 show that with grain of relatively low moisture content (16·7 and 17·0 %, 1955 crop [7*a*(ii)] and [6*a*]) although the oxygen concentration fell to 0·1 % within the first fortnight of storage, this low value was not maintained during the ensuing winter, when the concentrations recorded, although still low, were generally between 0·3 and 0·6 %. As the temperature rose during the following spring (1956) there was a slight fall in oxygen concentration in the one bin of grain of relatively low moisture content (wheat at 17·0 % moisture [6*a*]) remaining in store, followed by a small rise again the following winter and a later fall (summer 1957). This suggests that aerobic organisms were present and able to use up during the warmer weather any oxygen that entered by diffusion or leakage, but that during the winters the temperature was too low for this activity to take place. At high moisture contents (19 % and above) the very low value (0·1–0·2 %) was generally maintained throughout the year.

Effect of filling a container with carbon dioxide before sealing

A preliminary test in 4 lb. jars suggested that displacing the intergranular air by carbon dioxide before sealing, by allowing solid CO_2 to evaporate, might result in less severe changes in the grain than if the container were sealed filled with normal air and metabolic changes allowed to take place.

Accordingly, in the second season, one of the 10-ton bins [4*a*(ii)] was flushed out with carbon dioxide gas from cylinders before sealing, so that the intergranular concentration was over 97 %. It had been intended that the wheat in this bin should be at least as damp as that in the 'control' bin without added carbon dioxide. Unfortunately, a period of very wet weather between the filling of the first and second bins resulted in the grain in the second [4*a*(i)] having a higher moisture content than in that flushed out with carbon dioxide (22·7 % compared with 18·9 %).

It was therefore not possible to compare directly the grain in the two bins, but the general impression was that the addition of the carbon dioxide had little if any advantageous effect.

The high initial concentration of carbon dioxide was not maintained (Table 4), presumably due to absorption by the grain or to diffusion through the rubber gaskets.

Changes in gas concentration after opening the bins

The effect on the composition of the intergranular air of opening a bin at intervals and withdrawing a small quantity of grain, as might be done in practice, was investigated, in particular with a bin of wheat at 22·7 % moisture content [4*a*(i)]. At each opening ½–1 ton of grain was run out, the lower cover being removed for 30–45 min., and the top manhole cover usually loosened or temporarily removed. The bin was first opened in November 1954, after 11 weeks' storage, when the carbon dioxide concentration at both the top and bottom of the bin was the same and had been constant at about 84 % for some weeks, while the oxygen concentration was about 0·1 %. Immediately after resealing there was (Fig. 5) a decrease in the concentration of carbon dioxide, particularly at the top of the bin, but during the next few days the concentration at the top rose gradually and that at the bottom fell slightly, until they were equal to each other again but less than before opening. This sequence was repeated after each opening, approximately 2-monthly, so that the final concentration, about 60 %, after four openings was appreciably lower than the value of 84 % before the first opening.

A similar but converse effect occurred as regards oxygen concentration, which each time rose at the top of the bin to between 3 and 5 %, according to the length of time the bin had remained unsealed; the concentration at the bottom also rose slightly, but to a lesser extent. After resealing, after equalizing throughout the bin, the oxygen concentration then proceeded to fall, in a few days, to the usual minimum level of 0·1 %. This indicated that there were still present some aerobic organisms capable of becoming active if given a supply of oxygen. The fall was more rapid after the first opening than after later openings in January, April and May 1955. This may have been due to increasing length of storage having killed more of the aerobic micro-organisms, or, more probably, to the lower temperature of the grain mass at the later openings, 7–12° C. compared to 16° C. in November 1954, retarding utilization of the oxygen.

Another bin, containing wheat of 23·2 % moisture content [3*a*] and opened for the first time in February 1955 after 81 weeks' storage, showed the same general changes. As this bin was unsealed for a longer time at the first opening, the oxygen rose to a higher value, 18 % at the top and 6 % at the bottom, equalizing in a short time to 7 %. In this bin, however, the consumption of oxygen after resealing was much delayed, the concentration remaining more or less constant at about 6 % for 3½ months. After this it fell more or less suddenly to a low value. Throughout the time that the oxygen concentration had remained static the temperature of the grain was below 10° C.; the sudden consumption of oxygen corresponded to the temperature rising to 11° C. and above.

Similar results, as regards gas changes, were observed in a bin of barley of 19·2 % moisture content [7*a*(i)], opened for the first time in March 1957 after 80 weeks' storage. The carbon dioxide concentration, 58 % before opening, had equalized at the top and bottom of the bin at about 55 % 1 week after resealing, and the oxygen concentration was then uniformly about 2 %. It remained at this

relatively high value for several weeks, and then fell slowly to about 1·3 %. The bin was then (early May 1957) opened for a second time, and after resealing the carbon dioxide settled at about 46 % and the oxygen again at about 2 %. It was not until the end of May that the oxygen concentration showed any appreciable fall, after which time, when the temperature at the centre of the grain mass had risen to 11° C., the concentration decreased steadily, reaching the normal minimum of 0·1 % by mid-July.

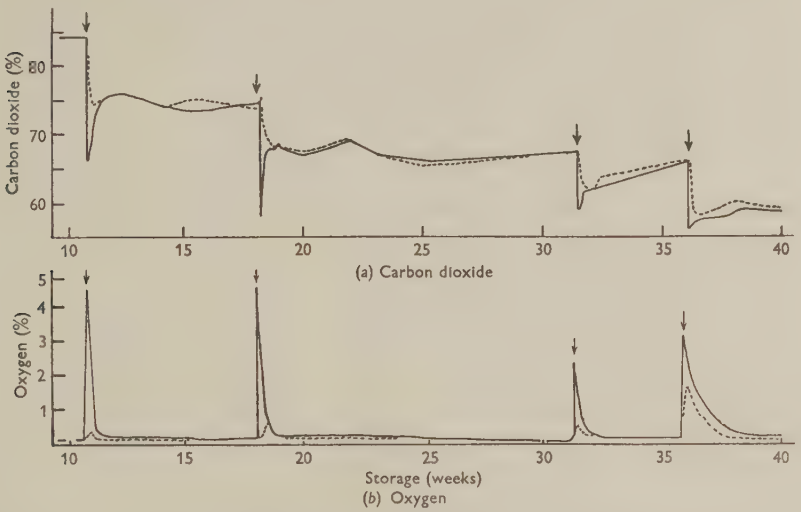

Fig. 5. Changes in the composition of the intergranular air in a 10-ton bin after successive openings (arrowed).

Thus it seems that the aerobic organisms associated with the grain are not all killed by prolonged airtight storage at a high moisture content, but that some remain alive but inactive, their growth when air is later admitted being dependent on the temperature being above a certain minimum value, apparently about 10° C. or a little above.

Respiratory quotient

Examination of the figures for concentrations of carbon dioxide and the associated oxygen, where available, shows that at all moisture levels while aerobic activity prevailed the volume of carbon dioxide produced was less than the oxygen consumed, i.e. the respiratory quotient (R.Q.) was less than unity. The results for a test using 2-gallon cans [5*d*] will be discussed in this connexion, for they are the most complete in this respect. The values (Table 5) of carbon dioxide produced plotted against oxygen consumed fall on a straight line giving an apparent R.Q. of 0·60 (Fig. 6). The rise at the end corresponds to the approach of anaerobic conditions.

At most moisture levels there was a progressive rise in the cumulative R.Q. after a drop from an initially slightly higher value (Table 5). Exceptions to this were the two wettest samples (24·4 and 21·8 % moisture content), where completely anaerobic conditions were reached very quickly, in 2 and 4 days, respectively, and in the driest grain (17·9 % moisture) which was anomalous in having an exceptionally low initial R.Q.

The reason for the consistently low apparent respiratory quotient is not clear. It has been observed by other workers, including Urion & Chapon (1955) and

TABLE 5. *Respiratory quotient in relation to moisture content and length of storage (2-gallon cans, 15° C.) [5d]*

Moisture content (%)	Storage (days)	Concentration of CO_2 (%)	Concentration of O_2 (%)	O_2 consumed* (%)	R.Q. (cumulative)
24·4	1	5·1	9·1	11·4	0·45
	2	11·8	0·2	20·3	0·58
21·8	2	6·3	9·4	11·1	0·57
	3	9·5	3·7	16·8	0·56
	4	12·2	0·2	20·3	0·60
20·3	2	2·3	17·2	3·3	0·69
	3	3·9	14·5	6·2	0·62
	6	6·5	9·0	11·5	0·57
	8	11·6	1·2	19·3	0·60
	9	12·6	0·3	20·2	0·62
	10	12·9	0·3	20·2	0·64
19·5	3	2·3	17·2	3·3	0·67
	6	5·1	12·1	8·4	0·61
	8	7·2	8·6	11·9	0·61
	11	10·0	4·3	16·2	0·62
	15	12·8	0·3	20·2	0·65
18·7	3	0·9	19·5	1·0	0·89
	6	2·1	16·7	3·8	0·54
	9	3·0	15·3	5·2	0·58
	15	5·1	12·2	8·3	0·61
	18	6·0	10·7	9·8	0·61
	22	8·0	7·6	12·9	0·62
	25	9·3	5·4	15·1	0·62
	28	10·3	4·1	16·4	0·63
	31	12·4	0·7	19·8	0·63
	34	13·1	0·3	20·2	0·65
17·9	8	1·4	17·5	3·0	0·46
	11	1·9	16·9	3·6	0·51
	16	2·4	15·6	4·9	0·48
	20	3·1	14·6	5·9	0·52
	23	3·8	13·4	7·1	0·54
	28	4·6	12·5	8·0	0·57
	35	5·4	10·9	9·6	0·56
	41	7·5	7·9	12·6	0·59
	49	9·3	5·2	15·3	0·61
	58	10·7	2·9	17·6	0·61
	65	13·1	0·5	20·0	0·65
	71	13·5	0·3	20·2	0·67

* Assuming normal atmospheric concentration as 20·5 %.

James & James (1940), whose much quoted value of 0·64 for dormant barley of unspecified moisture content seems to have become generally accepted without further confirmation.

Various possible explanations may be given. It may be that substances other than carbohydrates, such as fats, are being broken down in the grain, when a R.Q. of about 0·7 would be expected, as opposed to 1·0 from carbohydrates. Another suggestion is that some of the carbon dioxide produced is being absorbed physically by the grain. It is possible that the low R.Q. may be due to the metabolism of the

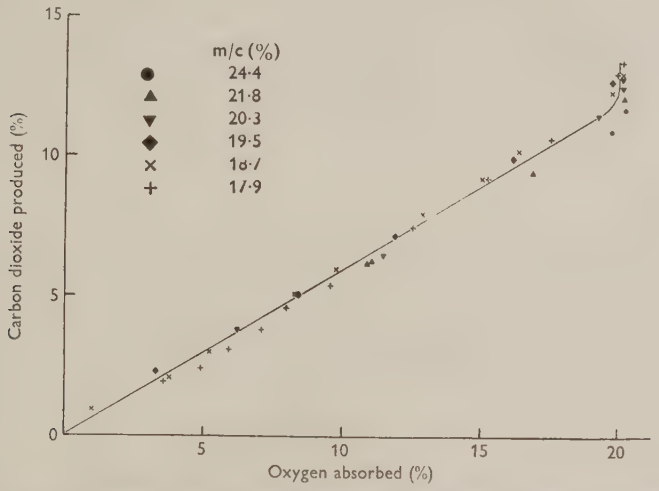

Fig. 6. Relation between carbon dioxide produced and oxygen absorbed in wheat stored hermetically in 2-gallon cans at the various moisture contents indicated.

micro-organisms present. According to Milner, Christensen & Geddes (1947*a*, *b*), fungal metabolism on wheat of moisture contents high enough to support the growth of moulds is characterized by an R.Q. of less than unity. There are very few data in the literature on the R.Q. of fungi as such. Hawker (1950) quotes various workers finding a quotient of approximately unity for a few species of fungi. Most of the statements on gas exchange relate to one substrate only, but it is known that the R.Q. can be altered by growing on another substrate.

There has, however, been very little work done on the respiratory quotient of resting grain, and it is hoped to investigate this subject further, not necessarily only under hermetic conditions resulting in complete utilization of oxygen.

Pressure changes inside containers

As would be expected, in the containers where appreciable production of carbon dioxide took place in the first few weeks of storage, there was considerable increase in the internal pressure. In the preserving jars and cans this pressure was not

measured, nor was it relieved by letting out air. It can be assumed that in those containers where the carbon dioxide concentration rose to 90–95% the internal air pressure amounted to several atmospheres. The preserving jars stood up to these high internal pressures very well, but for some of the cans they proved excessive, the cans then failed to become 'blown', but leaked at the seams and allowed the extra gas produced to escape.

The pressure in the 10-ton bins was studied in some detail. The results, as averages of 4-weekly periods, are given in Fig. 7. In the bins containing high-moisture grain [3*a*, 4*a*(i) and 7*a*(i)] there was appreciable increase in pressure during the period immediately after harvest, when carbon dioxide was being produced very rapidly. In 1953 [grain 3*a*], when the air and grain temperatures were high, about 20 and 30° C., respectively, and when carbon dioxide production was very rapid indeed, the lower closures of the bins were slackened and for a few minutes every day for the first few weeks, to allow excess gas to escape. The pressures

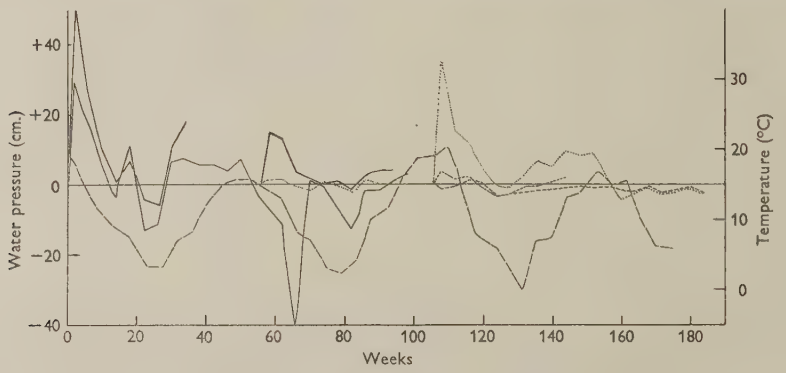

Fig. 7. Pressures in 10-ton bins containing hermetically stored grain. —— at 23% moisture content; .... at 19%; ---- at 17%. ——— temperature at London Airport.

recorded for this period do not therefore reflect the pressures which might have been attained. In other bins the production of carbon dioxide was more gradual, and release of gas was necessary on only one or two occasions, so that the pressures shown do not differ greatly from what would have developed in unopened bins.

With grain of lower moisture content there was less build-up of pressure inside the bins, although in 1955 the barley of 16·7% moisture content [7*a*(ii)] showed a development of slightly increased pressure in the period after harvest. Wheat of this season of 17·0% moisture content [6*a*] showed little pressure change from atmospheric, and the rate of production of carbon dioxide was also relatively slow. The bin filled in 1954 with wheat containing 18·9% [4*a*(ii)] was flushed out, after filling, with carbon dioxide gas from cylinders, so that there was a high concentration, about 97%, of this gas from the start; there was less post-harvest respiration, so the pressure changes were small.

After the first period of storage, as respiratory activity decreased, the pressure

ceased to rise. The pressures in the bins then varied considerably from day to day, and mainly reflected the barometric pressure and the temperature changes in the small free air space above the grain. Thus the monthly means tended to follow the pattern of the mean air temperatures, also plotted in Fig 7, falling in winter and rising in summer. Apart from a sudden fall in pressure in the bin filled in 1953 during the early winter of 1954, just before the Dutch barn previously mentioned was completed, and which resulted in a pressure low enough to necessitate addition of carbon dioxide gas to restore it to near-atmospheric again, the pressures did not change excessively during subsequent seasons.

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THE MANNER OF TRANSMISSION OF SOME BARLEY YELLOW-DWARF VIRUSES BY DIFFERENT APHID SPECIES

By MARION A. WATSON and T. MULLIGAN

Rothamsted Experimental Station, Harpenden, Herts

Some barley yellow-dwarf (BYD) viruses isolated from cereal crops in Great Britain were transmitted by *Rhopalosiphum padi*, L. and others were not. *Sitobion fragariae* (Walker), *S. avenae* (Fabricius), and *Metopolophium dirhodum* (Walker) all transmitted viruses of both types, but they usually transmitted those of which *Rhopalosiphum* was a vector less readily than did *R. padi*. The transmissibility of a virus by a given aphid species was not affected by transmission with another, less efficient, vector species. *Neomyzus circumflexus* (Buckt.) and *Rhopalosiphum maidis* (Fitch) transmitted the few viruses with which they were tested.

A few *R. padi* acquired virus from infected leaves during 30 min. feeding and inoculated healthy seedlings during 15 min. feeding, but the minimum total time taken to acquire and transmit was 10 hr. and 32 hr. were needed for about half the aphids that were able to acquire and transmit virus to do so. This may indicate the existence of a short latent period of the virus in the vector, although the evidence is not conclusive. The times spent on infected plants influenced the results more than those spent on healthy ones; many transmissions occurred with short feeding times on healthy plants so long as the time spent on infected leaves was long, but the reverse was not true. Nymphs of *R. padi* that moulted after they left infected plants on which they fed long enough to become infective, infected slightly fewer plants than adults fed for the same times.

Watson & Mulligan (1960) compared the host ranges of two barley yellow-dwarf viruses, one virulent (KV), the other avirulent (RV) and described how they interact in host plants and vectors and how they affect yields of cereals. The present paper compares the behaviour of the same two viruses when transmitted in varying conditions by *Rhopalosiphum padi* L. and describes the transmission of other British isolates of BYDV by different species of aphids.

Materials and methods

The viruses were isolated from plants or leaves collected in the field, by feeding virus-free aphids of one or more species first on the infected leaves and then on healthy test seedlings of Blenda oat. The methods of handling the aphids and maintaining them in virus-free culture have previously been described (Watson & Mulligan, 1960). When a virus was being tested with two different aphids halves of the same infected leaves were used as sources of virus (Rochow, 1958*b*).

The viruses are distinguished in this paper by the district from which they came. Two isolates from Rothamsted farm are called RV 54 and RV 59, the years in which they were isolated. Other isolates were from Kent (KV); Harlech, North Wales (HV); Bristol (BV); Hexton, nr. Luton, Beds (XV); Woburn Experimental Farm, Beds (WV).

The aphids used were originally collected in the field, and virus-free colonies made when this was necessary, by removing newly born nymphs to healthy cereal plants. They were identified by Dr D. Hille Ris Lambers or Dr H. L. G. Stroyan as *Rhopalosiphum padi* L., the bird-cherry aphid (Rogerson, 1947); *Sitobion fragariae* (Walker), the rubus aphid; *Metopolophium dirhodum* (Walker), the rose-grain aphid; *Sitobion avenae* (or *Macrosiphum avenae*) (Fab.), the grain aphid; *Neomyzus circumflexus* (Buckt.) and *Rhopalosiphum maidis* (Fitch), the corn-leaf aphid. The common names are taken from Thomas, 1948.

Oswald & Houston (1951, 1953) used aphids identified as *R. prunifolii* (Fitch), the apple-grain aphid, *Macrosiphum granarium* (Kirby), the English grain aphid; *Mac. dirhodum* (Walker), the grass aphid; *Toxoptera* (now *Schizaphis*) *graminum* (Rondani), the greenbug; and *Rhopalosiphum maidis* (Fitch), the corn-leaf aphid. Most American workers have used the same species, but *R. prunifolii* was referred to as *R. fitchii* (Sand) by Toko & Bruehl (1956) and Rochow (1958*a*, *b*).

Aphids sent by H. Jedlinski, Illinois, and Dr Rochow, New York, were identified by Dr Stroyan as *R. padi* (*R. fitchii*) and *S. avenae* (*Macrosiphum granarium*). *Macrosiphum dirhodum* is an accepted synonym for *Metopolophium dirhodum* although *Metopolophium* is not a subgenus of *Macrosiphum*, as is *Sitobion* (Hille Ris Lambers, 1947). *Rhopalosiphum maidis* has the same name in Europe and in America, and the greenbug is not found in Europe. *S. fragariae* and *N. circumflexus* have not been tested as vectors in America, but otherwise the vectors of BYD viruses appear to be the same as in Europe although they are known by different names.

RESULTS

Transmission of different BYD viruses by different aphid species

Toko & Bruehl, 1956, 'observed a partial superiority in transmission of a single Washington strain of BYDV' by *R. fitchii* over that by *Macrosiphum granarium*. These authors (1959) and Rochow (1958*a*, *b*), have since shown that there are two BYD viruses in the U.S.A. specifically transmitted by different aphid vectors. This is also true in Great Britain.

Table 1 shows that different viruses were transmitted exclusively or preferentially by different species of aphids. RV 54, which was transmitted for a year with some difficulty by *S. fragariae* and *Metopolophium dirhodum*, was much more efficiently transmitted later by *R. padi*. *S. avenae* and *N. circumflexus* also transmitted RV 54 less readily than did *R. padi*. HV was efficiently transmitted by *R. padi*, by *S. avenae* and *N. circumflexus*, but less efficiently by *S. fragariae*.

KV and HV were equally readily transmitted by *R. padi*, but KV was more efficiently transmitted by *S. fragariae* than by *S. avenae*. BV was transmitted like KV, although it caused more severe symptoms in Blenda oat.

XV was first observed in a late-sown crop of oats, var. Pendek, undersown with S. 23 perennial rye-grass. The oats showed typical bright red discoloration and stunting, but no virus was transmitted from them by *R. padi*. In the next year *Metopolophium dirhodum*, which was present on the cereals in both years, trans-

mitted virus from rye-grass and yellowed barley in a neighbouring field; *S. fragariae* and *S. avenae* also transmitted, but *R. padi* has never done so. XV, therefore, seems to be the equivalent of the viruses recognized in Washington and New York which are transmissible by *Macrosiphum granarium* but not by *R. fitchii* (Toko & Bruehl, 1956, 1959; Rochow, 1959*a*).

The second Rothamsted culture, RV 59, probably originally contained two viruses, for both *R. padi* and *S. avenae* transmitted from the original source,

TABLE 1. *Transmission of BYDV isolates by different aphid species*

	Vectors*					
Viruses and dates	*R. padi*	*S. avenae*	*S. fragariae*	*M. dirhodum*	*R. maidis*	*N. circumflexus*
RV 1954	—	—	**15/30**†‡	5/13†‡	—	7/16†‡
1955	**16/16**‡	—	9/16	0/6	—	—
1956	**26/31**‡	—	0/20	1/26	—	2/20
1959	13/13	0/15	0/15	0/15	—	—
Average % in 1959	100	0	0	0	—	—
HV 1957	**9/9**	—	—	—	—	—
	30/30	—	10/30†	—	—	**30/30**†
	10/10	40/40	—	—	—	—
Average % in 1957	100	100	33	—	—	100
KV 1958	**6/6**‡	—	—	17/26†	—	—
1959	**29/30**	0/30	**22/30**	**5/30**	—	—
	15/15	**3/22**	6/23	8/23	—	—
	20/20	1/33	—	—	—	—
Average % in 1959	99	5	51	25	—	—
BV 1958‡	**10/10**‡	—	—	—	—	—
1959	**29/30**	**2/30**	**21/30**	**7/30**	—	—
	—	**1/8**	**1/8**	**5/8**	—	—
	15/15	0/15	**2/15**	0/15	—	—
	20/20	**1/30**	**4/10**	—	—	—
1960	11/12	0/12	1/12	—	—	—
Average % in 1959	98	4	45	22	—	—
XV 1958	0/25	—	—	—	—	—
1959	0/30	—	—	**32/32**	—	—
	0/60	8/30	16/30	33/55	—	—
Average % in 1959	0	27	53	75	—	—
RV 1959	15/18	**19/19**	—	—	—	—
	0/60	33/70	—	—	—	—
Average % *S. avenae* isolate	0	47	—	—	—	—
WV 1959	—	—	—	**4/4**	3/5	—
	0/15	—	—	3/15	—	—
Average % *M. dirhodum* isolate	0	—	—	20	—	—

* = number of plants infected over number tested.
† = many aphids used in transfer; all others 5 or 10.
‡ = several transfers by the same vector before this one.
Bold figures = source of virus for tests shown in next row, all columns, unless joined by bracket, when the figure represents the source of virus for the test shown only immediately beneath it.

whereas the virus isolated by *S. avenae* was later transmitted only by *S. avenae* and not by *R. padi*.

WV was isolated from a barley plant growing among sugar beet at Woburn Experimental Farm. *Metopolophium dirhodum* and *R. maidis* were multiplying on the plant and both aphids transmitted virus from it, but only the one transmitted by *M. dirhodum* was further studied. It was not transmitted by *R. padi*, but *R. padi* transmitted virus from infected rye found at Woburn in the same year, so more than one type of virus was there, as at Rothamsted. In contrast at Hexton, about 10 miles from Woburn, only one virus was found; several efforts to transmit viruses from different sources by *R. padi* failed.

As in America the viruses in Britain seem to fall into two groups, one transmitted by *R. padi*, and also with varying efficiency by the *Sitobion* spp. and *M. dirhodum*, and a group not transmitted by *R. padi*, but by the *Sitobion* spp. and *M. dirhodum*. These aphids belong to the subtribe Macrosiphina erected by Theobald (1926), which provides a convenient collective title for the second group of viruses. They seem to have common vector abilities which they do not share with *R. padi*, although they can transmit the group of viruses transmitted by *R. padi*. Observations by Rochow (1959*b*) suggest that *R. maidis* and *Schizaphis graminum* may exhibit vector specificity different from that of the other aphid species. *Neomyzus circumflexus* transmits viruses of the *R. padi* group but has not been tested with the other.

Within the groups that are transmitted by different vectors there are viruses that differ in virulence. Viruses transmitted by different vectors or of different virulence, do not protect plants against each other. (Rochow, 1958*b*; Watson & Mulligan, 1960). There is no evidence that they are related strains and their exact relationships are unknown.

Table 1 is arranged to show how the isolates were subcultured. Brackets vertically connecting two values show that the infected plants represented in the upper entry were used as source of virus to infect those in the next row of the table, but not for all in that row. Entries in heavy type, but without vertical brackets, represent the source of virus for all subsequent transmissions.

This was done not only because some of the cultures obtained from the field contained viruses transmitted by different vectors, but also because vector specificity may have changed during the experimental transfers. Virus that originally had several vectors might have lost the ability to be transmitted by one or more of them as a result of being subcultured continuously by other species of aphids.

Although the ability of the *R. padi* group of viruses to be transmitted by Macrosiphina aphids declined slightly, this was not a result of transmission by *R. padi*, because Macrosiphina aphids usually transmitted *R. padi* isolates more successfully than those isolated by themselves. For instance, *S. fragariae* transmitted BV and KV isolated by *R. padi* to 70% of the plants fed on, whereas the virus isolated by *S. fragariae* was transmitted by *S. fragariae* to 25 and 15%, respectively, of the test plants in the next subculture.

Effect of different feeding times on infected and healthy plants

Table 2 shows the results of feeding *R. padi* on leaves infected with RV 54 and KV for 2, 8, 24, or 48 hr. and then on healthy test seedlings for the same times, so that the times were tested in all combinations. With RV the experiment was repeated on five occasions using seven plants per treatment and with KV on four occasions using five plants per treatment. Five aphids were used per test plant.

TABLE 2. *Effect of varying feeding times on infected and healthy plants on transmission to* Rhopalosiphum padi *of RV and KV*

Time on healthy plants	Time on infected plants				
	2 hr.	8 hr.	24 hr.	48 hr.	Total
	RV (infected out of thirty-five plants)				
2 hr.	0	1	11	31	43
8 hr.	0	1	15	29	45
24 hr.	3	16	23	33	75
48 hr.	13	20	30	33	96
Total	16	38	79	126	
	KV (infected out of twenty plants)				
2 hr.	0	1	7	15	23
8 hr.	1	2	13	18	34
24 hr.	0	8	17	19	44
48 hr.	10	10	19	20	59
Total	11	21	56	72	

Neither virus was transmitted when the aphids fed on infected and healthy plants for 2 hr. each. Even with total times of 10 hr. infections were few, and occurred mainly when the longer times were spent on the infected plants. When the total feeding times were 26 or 32 hr., infections were more numerous, but the times on the infected plants still influenced the results more than did those on the healthy ones.

Transmission increased with increasing feeding times on healthy plants for all times of feeding on the infected ones, except for RV given 48 hr. infection feeding. Theoretically longer test feeding of aphids fed for 2, 8 and 24 hr. on infected plants might have given still more transmissions, but this is unlikely in view of Rochow's results discussed on pp. 718–19. Probably the infectivity of aphids detected after feeding for 48 hr. on test plants, reflects the total number that acquired virus during their feeds on infected plants. If so the probability that virus will be acquired by a single aphid fed for 2 hr. on infected plants can be calculated to be about $P = 0{\cdot}10$ for RV 54 and $P = 0{\cdot}13$ for KV. About 50% of aphids acquired virus in 24 hr. The number that acquired it in 48 hr. cannot be calculated from this data; it could have been from 65 to 100% of the total population, but from the general trend of curves plotted from the data most of the aphids that could acquire virus had done so.

Only a third of the infective aphids transmitted virus to healthy plants during 2 hr. feeding when they had fed for 24 hr. on infected plants, so the chance of their transmitting in 2 hr. after only 2 hr. on infected plants was probably small and could have been missed.

Exp. 2 was therefore made using large numbers of *R. padi* which were fed on infected plants for 2 hr. and then cultured to healthy plants in groups of twenty-five aphids. On twenty of these plants they were allowed to feed for 2 hr. and on another fifteen, for 24 hr. Those fed for 2 hr. did not infect and those fed for 24 hr. infected all test plants. With twenty-five aphids per plant the probability that all fifteen plants fed on for 24 hr. would become infected was $P = 0{\cdot}21$, so at least 21 % of the aphids had acquired virus. If transmission increases even slowly from zero with time of feeding on healthy plants, several of the plants fed on by the aphids for 2 hr. should have become infected. The absence of these transmissions implies that aphids which had acquired virus were unable to transmit for at least 2 hr. after starting to feed on healthy plants.

A third experiment was made to detect the minimum time in which *R. padi* could acquire and transmit KV and BV. The two viruses were tested on different occasions some months apart. Aphids were either fed on infected leaves for 15, 30, 60 or 120 min. and then on healthy plants for 48 hr., or on infected leaves for 48 hr. and then on healthy plants for 15, 30, 60 or 120 min. There were ten plants per treatment and five aphids per plant.

TABLE 3. *Acquisition and transmission of KV and BV by* Rhopalosiphum padi *in short feeding times. Five aphids per test seedling, ten seedlings tested in two separate tests of five* (Exp. 2)

		Times of feeding				
		15 min.	30 min.	60 min.	120 min.	Total
Time on infected leaves	KV	0	1	0	1	2
(48 hr. test feed)	BV	0	2	4	4	10
Time on test plants	KV	2	4	8	8	22
(48 hr. infection feed)	BV	6	6	9	10	31

Table 3 shows that both viruses were transmitted after a minimum of 30 min. feeding on infected and 15 min. on healthy plants when the complementary feed was long. Aphids fed on KV infected leaves for 2 hr. or less infected only two plants in 48 hr. feeding. BV was more efficiently transmitted, about 10 % of the population acquired virus during 1 or 2 hr. feeding on infected plants. The results resembled those of Exp. 1 rather than Exp. 2 in which 21 % of aphids acquired virus during 2 hr. feeding.

These data are difficult to interpret in terms of a possible latent or incubation period of the virus in the vector. There seems to be a short period when aphids, particularly those that contain very little virus, are unable to transmit after leaving the infected plants. This may represent the time taken for virus to pass from the alimentary canal to the situation from which it is injected into a plant. Some such

interval is to be expected, and obviously if it is short, it can only be measured for aphids that have fed for minimal times on infected plants. Hence it is likely to be exaggerated, because small quantities of virus may be transmitted in small infrequent doses, and variation in plant susceptibility is likely to be limiting when the amount of virus injected into the leaf is small.

It is obviously misleading merely to combine the 30 and 15 min. respectively, taken for aphids to acquire and transmit the first traces of virus as in Exp. 3 (Table 3) and subtract this from the 10 hr. minimum total time in which virus was able to be acquired and transmitted in Exp. 1 (Table 2). The difference of 9·25 hr. might be part of a latent period, but also depends on other factors that probably could completely account for it. The dominant factor in transmission of BYDV seems to be the time taken to acquire virus and not that which elapses between acquiring and transmitting it. Similar results with other aphid-transmitted viruses are often assumed to demonstrate the existence of a latent period but usually they fail to do so conclusively.

The passage of BYDV through the moult

Young nymphs of *R. padi* were fed for 2 days on KV-infected leaves and then removed to seedlings of *Arrhenatherum elatior*, which is immune to the virus. Adults were fed for similar times on infected leaves and then for 3 days on the immune host. During the time the nymphs were on the immune host they moulted, usually into 4th-stage nymphs or adults, and were removed to healthy oat seedlings in groups of five. Adults were similarly removed to healthy seedlings at the end of their period on immune hosts. In three trials the moulted aphids infected 2/7, 4/5 and 2/5 of the test plants. The adults infected 4/5 and 5/5. The differences between these groups were significant at the 3% level according to the 'Exact' method (Fisher, 1936).

According to Toko & Bruehl, 1959, all instars of *R. fitchii*, (= *R. padi*), were equally efficient both in acquisition and transmission of BYD viruses, so in our experiment some virus seemed to have been lost by the aphids during the moult.

Similar results were recorded by Sylvester (1949), who found that the infective ability of nymphs carrying beet yellow-net virus was much reduced when they moulted. It might be postulated that gut walls are less adsorptive, or less pervious to virus, when the insects that ingest them are near to ecdysis, which is not surprising, because much of the lining of the gut is discarded with the integument. This would mean that virus is not lost during the moult but is less efficiently acquired by nymphs that are near to moulting.

Discussion

Many persistent viruses have more than one species of vector and there is often disparity between the vectors in the efficiency with which they transmit particular viruses, but the vector specificity of the BYD viruses is unusually complex. Rochow (1959*a*) compared it to that of potato yellow-dwarf virus (Black, 1944). Cucumber mosaic virus 1, a non-persistent virus, provides another example. One strain, obtained from spinach, is transmissible by *Myzus ascolonicus* and *Aphis*

gossypii but not by *Myzus persicae*, which transmits other strains of CMV 1 (Badami, 1958).

According to Rochow the two principal New York BYD viruses, AG and EG, do not exhibit complete vector specificity. In comprehensive tests vectors that usually transmit only one will occasionally transmit the other. He thinks this could be true of the Original and Washington isolates of Toko & Bruehl (1956, 1959), although it has not been demonstrated. We have not succeeded in transmitting XV, one of the group transmitted by Macrosiphina aphids with *R. padi*, although *R. padi*-transmitted viruses can be transmitted, with greater or less efficiency, by the Macrosiphina.

BYD viruses differ not only in vector specificity but also in virulence. Variations in virulence, like those of vector specificity, are diagnostic of some BYD viruses that do not protect against each other when successively inoculated to the same host plants (Allen, 1957; Watson & Mulligan, 1960). There may also be isolates of BYD that vary in virulence towards different species of Gramineae or their varieties, as some of Allen's 1957 data suggest. With so many permutations and combinations of characters it seems there may be a great number of BYD viruses, apparently all different and independent. However, they are so alike that it seems unreasonable to assume that they all have completely independent origins. Possibly the criteria by which relationship between plant viruses is usually decided are not appropriate.

Latent or incubation periods of persistent viruses in aphid vectors are often difficult to demonstrate because usually the times are short and become confused with the times taken for the insects to acquire virus and to infect plants. With BYD viruses, reports have varied even about the times of feeding on infected and healthy plants needed for aphids to transmit, and the interpretation of the results seems rather a matter of choice than of evidence.

Oswald & Houston (1951, 1953) reared their aphids on infected plants and then fed them on healthy ones for 3 days, to ensure infection. Allen (1957) quotes unpublished data by Freitag in which virus was acquired and transmitted during 5 min. feeds on infected and healthy plants, presumably used one at a time with the complementary feeding times prolonged.

Toko & Bruehl (1959) described experiments in which *R. fitchii* and *Macrosiphum granarium* were fed on infected and healthy plants for all combinations of a number of times varying from 10 min. to 3 days. Neither aphid transmitted when fed on infected plants for less than 24 hr., although seven shorter times, including 16 hr., were given. Aphids fed on infected plants for 24 and 48 hr. infected healthy plants they fed on for 4 or more hours, and the number infected increased with time as in our experiments. Toko & Bruehl seem to refer to this long delay in the aphids becoming infected as a latent period, but such results do not demonstrate a latent period because the virus was acquired so slowly that most of the apparent interval occurred before any was acquired.

Rochow (1959*a*) fed aphids singly for 1 day each on twelve or more successive healthy plants after feeds of 1, 24 or 72 hr. on infected ones. He gives full results

only for aphids that fed for 1 day, which infected three-quarters of the test plants fed on, but mentions that those fed on infected plants for 72 hr. infected more plants in succession, especially towards the end of the tests, and those fed for only 1 hr. infected test plants 'only occasionally', i.e. very much less than once every 24 hr. So the aphids varied, not only in the number that became infective, but in the amount of virus the infective ones acquired. Those fed for only short times on infected plants may have taken as long to infect the first healthy plants fed on as they did the succeeding ones. Probably they produced infective doses of virus only rarely. The latent period could be wholly accounted for by these factors or is impossible to measure because of them.

The arguments against multiplication of such viruses in aphids are even more cogent. Most aphids that have fed for short times on infected plants seem to contain little virus and rapidly lose infectivity when feeding on healthy plants. This is measurable and has been shown for several viruses transmitted by aphids and leafhoppers, including curly-top virus of sugar beet (Freitag, 1936) and leaf-roll virus of potato (Heinze, 1959). If virus multiplied, it should increase in the vectors and, if it increased, plants should be infected more frequently at later than at earlier tests, whereas the reverse usually occurs. Nevertheless, Stegwee & Ponsen (1958) showed that leaf-roll virus can be transmitted by injection through a series of aphids to a dilution of the original virus that precludes any other interpretation than that it multiplies in the vector. This could happen in other viruses with similar behaviour and be undetectable except by aphid-to-aphid transmission experiments. There are still unknown factors or those whose significance is misunderstood. Perhaps the vector specificity of BYD viruses depends on these factors, which may be related to susceptibility or penetrability of insect tissues, such as is postulated for races of leaf hoppers with varying ability to transmit maize streak virus (Storey, 1933) and wheat striate mosaic virus (Sinha, 1960).

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SUGAR-BEET YELLOWS: FURTHER STUDIES ON VIRUSES AND VIRUS STRAINS AND THEIR DISTRIBUTION IN EAST ANGLIA, 1958–59

By G. E. RUSSELL

Plant Breeding Institute, Maris Lane, Trumpington, Cambridge

Experiments have shown that, as in the years 1955–57, two yellowing viruses, beet yellows virus (SBYV) and sugar-beet mild yellowing virus (SBMYV), were present in commercial sugar-beet crops in East Anglia in 1958 and 1959. The evidence that they are not closely related viruses has been confirmed. In both years the prevalence of the two viruses was estimated by aphid transmissions from yellowed sugar-beet leaves to healthy sugar beet and *Chenopodium capitatum* seedlings in the glasshouse, and in 1959 additionally by examination of symptoms on field plants. SBMYV was more common than SBYV over the whole region in 1958, but in 1959 SBYV was slightly more prevalent than SBMYV. In both years SBYV was found more often in the southern than in the northern parts of the region. The results described in this paper suggest that breeding for tolerance to SBMYV may be at least as important economically in East Anglia as breeding for tolerance to SBYV. A wide range of SBYV strains was present in East Anglia in 1959, most of the strains being those which caused severe symptoms in sugar beet and *C. capitatum*.

Introduction

Russell (1958) reported that in 1955–57 two yellowing viruses were present in sugar-beet crops in East Anglia. One of these was beet yellows virus (SBYV) and the other was sugar-beet mild yellowing virus (SBMYV), and as they were apparently not closely related, it was unlikely that resistance or tolerance to one of them would necessarily be associated with tolerance to the other. It was therefore considered necessary, as an essential part of the virus yellows breeding programme being carried out at the Plant Breeding Institute, Cambridge, to assess, over a period of several years, the prevalence and distribution of SBYV and SBMYV in East Anglia, where yellowing viruses of sugar beet cause considerable yield losses in most years. In 1955, in the areas from which samples were taken, SBYV was more common than SBMYV; in 1956 this position was reversed. In 1957 the proportions of SBYV and SBMYV were roughly equal but SBMYV was more common than SBYV in the northern parts of East Anglia.

Similar surveys, but on a larger scale, have been carried out in 1958 and 1959. In 1959 an assessment of the relative proportions of SBYV and SBMYV was attempted by examining symptoms of plants in the field, in addition to the glasshouse and laboratory tests used in previous years. Attempts have also been made to group the strains of SBYV which have been obtained in the surveys, into closely related groups.

Materials and methods

Collection of source leaves

In both years, leaves which were yellow and brittle, symptoms of sugar-beet yellows infection, were collected from sugar-beet plants in commercial fields in East Anglia for testing in the laboratory and glasshouse. Usually one leaf was taken from three yellow plants growing in different parts of each field visited. As in previous years, in selecting the source plants, no attention was paid to symptoms other than yellowing or brittleness. The fields were selected generally for ease of access and not for the amount of infection visible, and they were always at least 5 miles apart.

In August 1958, leaves were collected from sixty-eight fields in different parts of East Anglia. In the following year 122 fields were visited in late July or early August, and a further sixteen fields in early September.

Glasshouse and laboratory tests

Aphid transmissions, using *Myzus persicae* (Sulz.), were made from the source leaves to healthy sugar beet and *Chenopodium capitatum* seedlings as soon as possible after collection, using the methods previously described (Russell, 1958). In some cases, sap from the leaves was tested for the presence of SBYV using antiserum prepared against a virulent strain of SBYV which produces severe symptoms on sugar beet and *C. capitatum*. The inoculated sugar-beet plants were examined after 14 days for the presence of vein-etch symptoms, and after a further 3–4 weeks for yellowing symptoms and necrotic spots on the older leaves. At the end of this period some of the plants which showed yellowing but no vein-etch or necrotic spots, were cross-inoculated with a virulent SBYV strain using ten viruliferous *Myzus persicae* per plant. Where vein-etch symptoms subsequently appeared in the young leaves of cross-inoculated plants, it was assumed that they had not previously been infected with SBYV.

C. capitatum is very sensitive to SBYV infection and within 7 days of inoculation with this virus, the centre leaves show severe distortion with vein-clearing and most infected plants die prematurely. SBMYV causes no discernible symptoms in this host and no virus has been recovered from inoculated plants. The plants inoculated from the source leaves were examined for SBYV symptoms 2 weeks after inoculation. Inoculations from leaves infected with SBYV produced symptoms on *C. capitatum* which varied from mild distortion of the centre leaves and slight stunting to very severe distortion and death of the host within a few weeks, according to the virulence of the SBYV strain or strains involved. These strains were assigned to five groups according to the severity of symptoms on *C. capitatum*, group 1 consisting of those strains which produced the mildest symptoms and group 5 of those which produced the most severe symptoms.

Sap from some of the source leaves was examined under the electron microscope for the presence of the characteristic beet yellows virus filaments and for particles specific to plants infected with SBMYV, using the negative staining technique of

Brenner & Horne (1959). The marked periodic structure of SBYV filaments enabled this virus to be distinguished very easily from other viruses (Horne, Russell & Trim, 1959).

Examination of symptoms of field plants

In 1959, in addition to the collection of source leaves, thirty plants with yellow and brittle older leaves were selected at random from different parts of each field visited, and examined for symptom differences. In the previous year it had been noticed that field plants infected only with SBMYV had leaves of a bright orange colour with no brown or red necrotic spots, and that the orange leaves from such plants were more often attacked by secondary fungal pathogens such as *Alternaria* spp. than were leaves from plants infected with SBYV. Leaves infected with SBYV only often showed vein-etch in the centre leaves if the infection was recent, and invariably showed brown or red necrotic spots on the old yellow leaves if the infection was well established. It was found that the field symptoms of the two viruses could readily be distinguished. Plants which presumably had become infected with both viruses showed a combination of both types of symptoms, such as necrotic spots on orange leaves, and it was often possible to see with which virus a plant had become infected first. In 1959 it was not uncommon to find plants with symptoms of an obviously long-standing SBMYV infection, but with vein-etch symptoms or necrotic spots in the younger leaves of a more recent SBYV infection. This ability to determine mixed infection of SBYV and SBMYV proved very valuable because these mixed infections were impossible to demonstrate using simple aphid-transmission experiments. The proportion of plants showing symptoms of both viruses was not assessed accurately in 1959, although this would have provided additional useful information; however, rough estimates on the extent of double-infections were made in most of the fields visited.

An assessment of the prevalence of SBYV and SBMYV based upon visual symptoms was made in 122 fields in July and early August. Another sixteen fields in Norfolk were visited in early September and again at the end of September to follow the spread of the two viruses within the crops.

For the purpose of the surveys in 1958 and 1959, East Anglia was divided into four areas: (1) Essex and Hertfordshire; (2) Suffolk and south-east Cambridgeshire; (3) Norfolk; (4) North-west Cambridgeshire, Isle of Ely, Huntingdonshire, Bedfordshire, Soke of Peterborough, Holland division of Lincolnshire.

Results

The number of plants infected with SBYV and with SBMYV in the samples collected, as estimated by transmission tests, is shown in Table 1. In 1958, SBMYV was more common than SBYV in all areas, more particularly in areas 1 and 4, although the differences between areas were not statistically significant ($\chi^2 = 5{\cdot}09$ with 3 D.F.). There was no evidence of significant differences between fields within areas. In July and early August 1959, SBYV was more prevalent than SBMYV in

areas 1 and 2 but less prevalent in areas 3 and 4, differences between the areas being highly significant ($\chi^2 = 33{\cdot}91$ with 3 D.F.). There were no significant differences between the transmission test results of areas 1 and 2 and none between those of

TABLE 1. *Results of aphid transmissions to glasshouse beet seedlings from field sugar-beet plants apparently infected with yellowing viruses:* 1958 *and* 1959

Date of collection	Type of symptoms produced on test seedlings	Virus	Number of transmissions from source plants producing SBYV or SBMYV symptoms: Area 1	Area 2	Area 3	Area 4	Total no. of plants tested	
August 1958	Vein-etch and yellowing	SBYV	13	38	18	10	79	204
	Yellowing but no etch	SBMYV	26	46	24	29	125	
Late July and early August 1959	Vein-etch and yellowing	SBYV	46	94	34	28	202	366
	Yellowing but not etch	SBMYV	14	53	44	53	164	
Early September 1959	Vein-etch and yellowing	SBYV	—	—	25	·		48
	Yellowing but no etch	SBMYV	—	—	23	—		

TABLE 2. *Estimation of prevalence of SBYV and SBMYV based on visual symptoms in the field,* 1959

Date of count	Type of symptom on field plant	Virus	Number of field plants examined showing SBYV or SBMYV symptoms: Area 1	Area 2	Area 3	Area 4	Total no. of plants examined	
Late July and early August 1959	Vein-etch or necrotic plots on old yellow leaves	SBYV	426*	802*	215*	271*	1714	3660
	Orange leaves, no vein-etch or necrotic spots	SBMYV	174	668	565	539	1946	
Early September 1959	Etch, necrotic spots on yellow leaves	SBYV	—	—	185*	—		480
	Orange leaves, no etch or necrotic spots	SBMYV	—	—	295	—		
Same fields as above in late Sept. 1959	Etch, necrotic spots on yellow leaves	SBYV	—	—	264*	—		480
	Orange leaves, no etch or necrotic spots	SBMYV	—	—	216	—		

* Many plants showing SBMYV symptoms as well.

areas 3 and 4, but the results of areas 1 and 2 combined were markedly different from those of areas 3 and 4 combined. As in the previous year, there were no significant differences between estimates from different fields in an area. In area 3 another collection of leaves was made at the beginning of September 1959 and this indicated that there had been an increase of SBYV infection in that area between early August and early September.

Estimates of the proportions of SBYV and SBMYV obtained by examination of

symptoms on field plants in 1959 (Table 2) were generally similar to those obtained from the results of transmission tests. To test the closeness of the agreement between the two methods, each field was assigned to one of five groups according to the number of plants examined which showed SBYV symptoms, and also to one of four groups according to the number of source plants in which SBYV was detected (Table 3). The association between the two methods measured in this way was very close, giving a χ^2 of 114 with 12 D.F.

TABLE 3. *Sugar-beet yellows: association between results of transmission tests and symptom counts in East Anglia*, 1959

Number of yellowed plants in a field which showed SBYV symptoms (thirty plants examined)	Number of yellowed plants in a field in which SBYV was detected by transmission (three plants sampled)				
	0	1	2	3	Total
0–5	19	3	2	0	24
6–11	5	17	18	2	42
12–17	3	7	11	5	26
18–23	0	4	7	13	24
24–30	0	0	6	16	22
Total	27	31	44	36	138

$\chi^2 = 113{\cdot}81$ (12 D.F.).

In the symptom counts made in late July and early August, SBYV was more common than SBMYV in areas 1 and 2 but not in areas 3 and 4 (Table 2). There were marked differences between the results of counts of individual fields within each of the four areas and an ordinary χ^2 test could not be used for testing differences between areas. To overcome this difficulty, a contingency table was arranged with columns representing areas and rows representing numbers of plants (grouped in intervals of fives) which showed SBYV symptoms in individual fields. A χ^2 test on the data arranged in this way gave a χ^2 of 37·14 with 6 D.F., indicating large significant differences between the symptom counts obtained from different areas. Counts of areas 3 and 4 were not significantly different from each other and differences between counts in areas 1 and 2 were barely significant, the most pronounced difference being between the combined results of areas 1 and 2 and those of areas 3 and 4. Using the visual method of assessment it was possible to note that in all areas, but particularly in areas 1 and 2, a large proportion of plants showing SBYV symptoms was also infected with SBMYV, the SBMYV infection having generally occurred earlier than the SBYV infection. Sixteen fields in area 3 were visited at the beginning of September and again at the end of the same month, the proportions of SBYV and SBMYV infections being estimated by eye. It was found (Table 2) that although the fields at the first visit had more plants which showed only SBMYV symptoms than SBYV symptoms, 3 weeks later plants showing SBYV symptoms were more common than plants showing only SBMYV symptoms. It was noted at the second count that a very large proportion of the plants showing SBYV symptoms was also showing symptoms of SBMYV. Five fields in area 4

which had been visited in early August and which had shown little SBYV infection, were revisited later in September, and in these there was not such a rapid increase in the number of plants showing SBYV symptoms between the two counts as there was in area 3, SBMYV accounting for 86 % of plants infected with yellowing viruses at the first count and for 73 % at the second count.

The glasshouse and laboratory tests confirmed earlier results that SBYV and SBMYV are not closely related. Transmissions from plants infected only with SBMYV never produced any visible symptoms in *C. capitatum*, whereas all strains of SBYV produced stunting and distortion of this host. In the electron microscope, the characteristic filamentous SBYV particles have never been seen in sap from plants infected only with SBMYV, but dissimilar particles, apparently specific to plants infected with SBMYV, have been noticed and these, which may be the infectious particles of SBMYV, are being examined further. Sap from plants infected with SBMYV consistently failed to react with antiserum prepared against SBYV. Plants infected with SBMYV were not protected against infection with SBYV; after cross-inoculation with SBYV, the plants infected with SBMYV showed SBYV symptoms as quickly and as severely as did plants which were previously not infected with SBMYV.

Table 4. *Percentages of SBYV strains from source leaves collected in* 1959, *assigned to each SBYV symptom group*

Group 1 very mild symptoms, group 5 very severe symptoms, on *C. capitatum*.

	Symptom group				
	1	2	3	4	5
Area 1	10	33	22	14	21
Area 2	11	10	12	28	39
Area 3	4	7	23	35	31
Area 4	11	13	14	26	36
Total in whole region	36	63	71	103	127

The *C. capitatum* plants inoculated from source leaves infected with SBYV in 1959, were grouped according to the severity of symptoms produced; Table 4 shows the proportions of the SBYV strains falling into each symptom group, which were obtained from each of the four areas. Of those source leaves which proved to be infected with SBYV in areas 2, 3 and 4, about two-thirds were infected with strains which caused very severe symptoms in *C. capitatum* (symptom groups 4 and 5); in area 1 only about one-third of the strains collected were in these two groups, the less virulent strains (symptom groups 1 and 2) accounting for nearly one-half of them. Area 3 had a smaller proportion of strains in groups 1 and 2 than did other areas. It was found generally that the SBYV strains causing the most severe symptoms in *C. capitatum* were those which caused severe symptoms in sugar beet, but there were apparent exceptions, some strains appearing more virulent in *C. capitatum* than in sugar beet. *C. capitatum* was used in this work because it is a more sensitive host plant than sugar beet and consequently differences in virulence are easier to distinguish.

Discussion

The results of transmissions from field sources in 1958 and 1959 confirm earlier results that sugar-beet yellows is a complex disease resulting from infection with either or both of the yellowing viruses SBYV and SBMYV. The proportions of the two viruses varied considerably at different times of the growing season, from year to year and from place to place in a particular year. In 1958 when SBMYV infection was twice as common as SBYV infection in the samples collected from the region as a whole, SBYV infection was diagnosed in nearly half the samples collected from areas 2 and 3, although the differences between areas were not statistically significant. In 1959, when SBYV was slightly more common than SBMYV over the whole region, SBMYV was the more prevalent virus in areas 3 and 4, these differences being highly significant. The transmission experiments of 1959 tended to underestimate the amount of SBMYV infection, particularly in areas 1 and 2, because these tests enabled only the presence of SBYV to be diagnosed when both viruses were present in the source leaves.

The development of a rapid and reliable method of visual assessment of the relative prevalence of the two viruses in a field enabled many more plants to be examined than previously and served as a useful check on the results of the transmission tests. On the whole, the results of the two methods agreed well in 1959; however, the visual assessment method is necessarily based on the examination of well-established symptoms and recent infections and cross-inoculations could not be detected, but the presence of both viruses in a plant could usually be detected without difficulty where symptoms of both viruses were well defined. In 1959, the results of symptom counts tended to underestimate the amount of SBYV in the crops visited, probably because spread of SBYV or the development of SBYV symptoms was still taking place in August and September and many of the plants infected with this virus were not showing plainly visible SBYV symptoms at the time of counting; aphids would, however, have been able to transmit SBYV from these plants before the appearance of symptoms. Estimates of the prevalence of SBYV and SBMYV based on symptom counts made in area 3 in late September 1959, agreed very closely with those based on the results of transmissions from sources obtained in the same fields a few weeks earlier, the inference being that symptoms of SBYV on plants infected with that virus during August did not become easily discernible until late September. One definite conclusion which could be drawn from the results of the 1959 survey was that there was a fairly general early SBMYV infection over the whole region, followed by a spread of SBYV from the south-east to the north-west.

For the plant breeder the type of information obtained in these experiments is of great importance because it shows that a resistant or tolerant sugar-beet variety must be resistant to SBYV and to SBMYV separately and also to both viruses together. These small-scale investigations have clearly shown the importance of SBMYV infection in East Anglia in 5 successive years, and in some years resistance to this virus would seem to be more important economically than resistance to

SBYV. In most years, a variety resistant or tolerant to SBYV but not to SBMYV, would be of very limited value in areas 3 and 4, where SBMYV has been more common than SBYV. It is obviously desirable that varieties should be resistant to both viruses and a programme of breeding for tolerance to SBMYV as well as to SBYV, is now in progress at Cambridge. Unpublished results of field trials at the Plant Breeding Institute, Cambridge have shown that tolerance to SBYV is not necessarily associated with tolerance to SBMYV, although a small proportion of breeding lines were tolerant to both viruses. There is also some evidence that there are differences between breeding lines in their tolerance to different strains of SBYV, and the information concerning the relative importance of the various SBYV strains and strain-complexes, afforded by these investigations, is proving very valuable.

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FACTORS AFFECTING THE SPREAD OF APHID-BORNE VIRUSES IN POTATO IN EASTERN SCOTLAND

III. EFFECTS OF PLANTING DATE, ROGUING AND AGE OF CROP ON THE SPREAD OF POTATO LEAF-ROLL AND *Y* VIRUSES

By C. H. CADMAN and J. CHAMBERS

Scottish Horticultural Research Institute, Invergowrie, Dundee

Experiments at Invergowrie, south-east Perthshire, showed that the extent of spread of potato leaf-roll and *Y* viruses varied from year to year and that virus *Y* consistently spread more than leaf roll. Most spread of Virus *Y* occurred before the end of June and of leaf-roll virus before the end of July. Both viruses spread slightly more in late- than in early-planted crops. When plants with leaf roll and already colonized by *Myzus persicae* were placed in a healthy crop of Majestic potatoes at intervals during the season, the amount of virus spread decreased rapidly with increasing age of the crop. Spread of leaf roll occurred in all of twenty-five 'seed' crops in different districts of eastern Scotland in 1955 but in only twenty out of thirty-six similar crops in 1956. Annual and regional differences in virus spread appear to reflect differences in the time at which migrant aphids reach potato crops in early summer and the rate at which infestation builds up in the crops.

Introduction

The environment of the eastern counties of Scotland obviously favours the production of 'seed' potatoes, and more than 60,000 acres are usually grown there. However, the facts that stocks have to be rogued each year and that outbreaks of leaf roll sometimes occur show that viruses spread in eastern Scotland. The experiments reported here were begun because there was no quantitative information on the spread of potato *Y* and leaf-roll viruses in Scottish 'seed' potato crops and because the occasional epidemics of leaf roll were not satisfactorily explained. There are two possible explanations for the spread of these aphid-borne viruses in potato crops: either the viruses are brought in by aphids dispersing from infected crops, or spread within crops occurs before infected plants are removed. In either event, spread of aphid-borne viruses must reflect the activities of aphids at a time when the crops are susceptible to infection.

The overwintering of the aphid vectors of potato *Y* and leaf-roll viruses and the pattern of aphid infestation of potato crops in eastern Scotland were described by Fisken (1959 *a*, *b*) and our results complement his. Fisken found that three species of aphids, *Myzus persicae* (Sulz.), *Macrosiphum euphorbiae* Thomas and *Aulacorthum solani* (Kltb.), commonly occur on potato crops throughout eastern Scotland. All three species overwinter as apterae on outdoor and protected market garden crops, chiefly in the Lothians and in the Moray Firth area, and fly to potato crops in early summer. Surveys during 1954, 1955 and 1956 showed that potato crops near to overwintering sites became colonized between mid-May and mid-June, whereas

those more distant remained free from aphids until the end of July or mid-August. Because aphids usually did not disperse from potato crops until late August or September, Fisken suggested that spread of leaf-roll and *Y* viruses probably depended on the time of the spring migration and the rate at which crops became infested by aphids. Our results support this idea and show that most spread of aphid-borne viruses occurs early in the season at a time when potato plants are highly susceptible to infection.

Material and methods

The techniques of experimentation used were similar to those developed at Rothamsted (Doncaster & Gregory, 1948; Gregory, 1948). Most of the information on virus spread was obtained by harvesting the tuber progenies from each of three or four plants on each side of and in the same drill as a virus-containing (infector) plant, growing these on the following year and recording the proportions of plants which showed symptoms of leaf roll or of infection by virus *Y*. Each group of six or eight plants is called an infector unit. Entire tuber progenies were always harvested and grown on, as preliminary tests showed that many progenies were only partially infected and that single-tuber samples gave an unreliable indication of virus spread.

For field experiments at Invergowrie, foundation stocks of Majestic were used in 1953, 1954, 1957 and 1958, foundation Kerr's Pink in 1955 and once-grown Kerr's Pink in 1956. Plants of King Edward containing potato *Y* or leaf-roll viruses were used as infectors in most experiments but leaf-roll-infected Arran Consul plants were used in two made in 1957 and 1958. In all experiments the tubers were planted 18 in. apart in drills spaced at 27 in. Each year, except where stated otherwise, tubers were planted during the second fortnight of April, and 50 % of plants emerged usually within 4–6 weeks of planting.

Experiments to find the rate of spread of leaf-roll virus in field crops were done by planting ten leaf-roll infectors randomly in each of the selected crops, harvesting ten infector units of six plants each and scoring the progenies for leaf-roll infections the following year. Of necessity, the field crops included a range of potato varieties, and infectors of varieties readily distinguishable from the experimental crop were always used. Potato crops are planted earlier in Midlothian than in north Angus and Perthshire and the infector tubers were usually put into each crop a few days after it was planted. Where they are known, the dates when the field crops were planted are shown in Table 6. Some crops had their haulms killed in late July or early August but most were allowed to mature. In either event, the tuber samples were dug before the crop was harvested and all were stored over winter at Invergowrie and grown there the following year for observation.

Results

Effect of time of planting on virus spread

Experiments were made each year from 1953–57, always with the same general design. There were forty-eight plots, each 11 plants × 5 drills (31 plants × 7 drills in 1953) with a centrally placed infector; half the plots contained virus *Y* and half contained leaf-roll infectors. Plots were planted during the 1st or 3rd weeks of April and the 1st or 3rd weeks of May. Each treatment was replicated six times and the treatments were distributed randomly among the forty-eight plots. Infector units of eight plants were harvested from each plot.

The 1955 experiment was not made as planned and the results from the plots planted with virus *Y* infectors in 1956 had to be rejected because of the high

Table 1. *Spread of potato Y and leaf-roll viruses in potato crops planted on different dates*

		Virus *Y*					Leaf-roll virus				
Indicator var.	Year	1st week April	3rd week April	1st week May	3rd week May	Total	1st week April	3rd week April	1st week May	3rd week May	Total
Majestic	1953	6*	9	13	9	37	5*	4	7	3	19
Majestic	1954	0	3	1	3	7	0	1	1	0	2
Kerr's Pink	1956	—	—	—	—	—	4	4	1	8	17
Majestic	1957	4	6	6	11	27	2	2	3	4	11
Total		10	18	20	23	72	11	11	12	15	49

* Number of plants infected out of 48.

Table 2. *Pooled frequencies of plants infected with Y or leaf-roll viruses at different distances from infectors in crops planted at different times*

	Virus *Y*					Leaf-roll virus				
	Plant position					Plant position				
Year	1	2	3	4	Total	1	2	3	4	Total
1953	20	13	2	2	37	11	5	2	1	19
1954	7	0	0	0	7	1	1	0	0	2
1956	—	—	—	—	—	9	4	2	2	17
1957	12	7	8	0	27	7	4	0	0	11
Total	39	20	10	2	71	28	14	4	3	49
Date of planting										
Early April	4	4	2	0	10	7	4	0	0	11
Mid-April	11	4	2	1	18	9	1	0	1	11
Early May	10	6	4	0	20	5	4	2	1	12
Mid-May	14	6	2	1	23	7	5	2	1	15
Total	39	20	10	2	71	28	14	4	3	49

incidence of this virus in the indicator crop, a foundation stock of Kerr's Pink grown at Invergowrie in 1955. Tables 1 and 2 summarize the results from the remaining experiments. Although new infections were too few for statistical analysis,

there is some indication that both viruses, particularly virus *Y*, spread more in late- than in early-planted plots. But these differences were small compared with those between viruses and between years. In the 3 years with comparable experiments, virus *Y* spread twice as much as leaf roll and both viruses spread little in 1954, a wet and cool summer.

The pooled frequencies of new infections (Table 2) show that plants adjacent to the infectors became infected oftener than those more distant. As this was true of both viruses in all years, the gradients suggest that infection spread from the infector plants and not from sources outside the crop.

Effect of time of roguing

Experiments with virus *Y* in 1953 and 1954, and with leaf-roll and *Y* viruses in 1956, compared the amount of virus spread in unrogued plots with that in plots from which infected plants were removed during the last week of June or the second week of July.

TABLE 3. *Effect of roguing on spread of Y and leaf-roll viruses*

		Treatment		
Year	Virus	Rogued late June	Rogued mid-July	Unrogued
1953	*Y*	42*	48	40
1954	*Y*	9	0	7
1956	*Y*	7	12	11
	LR	12	8	11

* Number of plants infected out of 192.

The 1953 and 1954 experiments each consisted of six randomized blocks of three plots, each 16 drills × 11 plants. The sixth plant in drills 4, 7, 10 and 13 of each plot was a King Edward plant containing virus *Y*. Four infector units of ten plants were harvested from each plot. The 1956 experiment consisted of thirty-six randomized plots, each 8 drills × 9 plants. The fifth plants in drills 3 and 6 of each plot were King Edward infectors: half the plots contained *Y* and half contained leaf-roll infectors. At lifting time, two infector units of eight plants each were harvested from each plot. The planting dates were 14–16 April and the roguing dates were 30 June and 13 July in 1953, 28 June and 12 July in 1954, and 25 June and 13 July in 1956. Roguing consisted in removing infected haulms and tubers with as little disturbance of the crop as possible. The pooled results (Table 3) suggest that both viruses spread early in the growing season because virus spread as much in plots rogued in late June as in unrogued plots.

This was confirmed by experiments in 1955 and 1957, when spread in unrogued plots was compared with that in plots rogued at weekly intervals during the growing season. A randomized block design comprising 144 plots was used in 1955, and a randomized plot lay-out comprising 120 plots in 1957, each plot being 16 drills × 9 plants and containing four infectors, the fifth plants in drills 4, 7, 10 and 13. Half the plots contained virus *Y* and half contained leaf-roll infectors, and four infector

units of eight plants were harvested from each plot. Roguing was done on nine successive weekly occasions, beginning 20 June in 1955, and on seven such occasions, beginning 24 June, in 1957. These treatments were replicated six times. In each experiment the remaining thirty-six plots were not rogued.

The results (Table 4) show that both *Y* and leaf-roll viruses spread early in the season: indeed, roguing during the last week of June in either 1955 or 1957 failed materially to decrease the spread of virus *Y* although it decreased the spread of leaf-roll virus. Both viruses spread more in 1955 than in 1957 but the figures suggest that most of the season's spread occurred earlier in 1957 than in 1955: the little spread of leaf-roll virus in the unrogued plots in 1957 is unexplained. Plants close to the infectors became infected oftener than those more distant and in each year virus *Y* spread at least twice as much and further than leaf roll. Thus, in 1955, 8·6 %, and in 1957, 5·9 % of plants harvested from plots containing leaf-roll in-

TABLE 4. *Pooled frequencies of plants infected with Y or leaf-roll viruses in plots of Kerr's Pink* (1955) *and Majestic* (1957) *potatoes rogued at weekly intervals*

Date rogued	Plant position 1	2	3	4	Total
	Kerr's Pink planted 25 April 1955				
20 June	6: 0*	2:0	3:0	5:0	16: 0
27 June	24: 2	15:1	13:1	11:2	63: 6
4 July	31: 4	24:2	13:1	13:1	81: 8
18 July	27: 4	20:3	14:4	8:2	69:13
25 July	31: 4	22:4	13:1	11:0	77: 9
1 August	34:14	19:8	11:4	10:6	74:32
8 August	37:10	19:8	10:6	8:3	74:27
15 August	37:14	27:5	17:2	10:1	91:22
Not rogued†	33:15	22:8	15:4	12:4	82:31
	Majestic planted 15 April 1957				
24 June	19: 5	9:1	8:0	6:0	42: 6
1 July	13: 7	11:3	3:2	4:1	31:13
8 July	18: 6	11:3	6:2	4:1	39:12
15 July	16: 5	10:1	5:2	6:1	37: 9
22 July	19: 8	11:4	6:4	5:1	41:17
29 July	21: 8	12:3	6:2	5:1	44:14
5 August	9:11	7:4	4:2	0:1	20:18
Not rogued†	13: 3	11:1	7:1	5:1	36: 6

* *Y*:*LR*, numbers of plants infected out of 48.
† Average of 18 plots divided by 3.

fectors were infected with virus *Y*, whereas only 0·7 % of plants from plots containing virus *Y* infectors became infected with leaf-roll virus. Particularly in 1955, cross-infections with virus *Y* occurred oftener among plants distant from the leaf-roll infectors. Because both leaf-roll infectors and the crop were initially free from virus *Y*, the figures probably indicate widespread dissemination of virus *Y* from the virus *Y* infectors. Another interesting feature of these experiments was the high proportion of plants which produced partially infected progenies. In 1955, 78 % and in 1957, 88 % of plants which became infected by virus *Y* produced partially

infected progenies: the corresponding proportions for leaf-roll infected plants were 60 and 45 %, respectively. The distribution of plants which produced totally infected progenies seemed unrelated either to the roguing treatment or to distance from the source of infection. Only 30 % of the total tubers harvested from the 1955 and 1957 experiments produced diseased plants.

Relation of age of crop to virus spread

The roguing experiments showed that *Y* and leaf-roll viruses spread early in the growing season, so experiments were made in 1957 and 1958 to find whether the spread of leaf roll in Majestic potato varied with the age of the crop. The design consisted of two equal strips of plots, each 8 drills × 9 plants, separated by twelve drills of healthy potatoes and surrounded by similar plants. Beginning 6 weeks after the crop was planted and at fortnightly intervals thereafter, the centre row of eight plants of one plot in each strip was replaced by eight leaf-roll-infected Arran Consul plants, each heavily infested by *Myzus persicae*. The treatments

TABLE 5. *Percentage (according to position) of Majestic plants infected with leaf-roll virus in plots exposed to infection at different times*

Date infectors exposed	Plant position					Totally infected
1957	1	2	3	4	Mean	progenies
24 June	96·8	96·9	78·6	53·6	81·5	66·4
8 July	100·0	93·5	74·2	36·7	76·1	35·0
22 July	84·4	50·0	26·7	6·5	41·9	12·8
5 August	18·8	6·5	0·0	0·0	6·3	0·0
19 August	0·0	0·0	0·0	0·0	0·0	0·0
No infectors*	2·6	1·3	2·5	1·9	2·1	0·0
1958						
27 June	96·9	90·6	81·2	48·4	79·3	55·9
11 July	78·1	60·7	32·3	19·4	47·6	19·7
25 July	78·1	28·1	3·2	0·0	27·3	7·2
11 August	18·8	9·7	0·0	0·0	7·1	0·0
No infectors†	0·0	0·0	0·8	2·4	0·8	0·0

* Mean of 10 plots. † Mean of 8 plots.

were arranged serially but in reverse order in each strip, so that plots which received infectors first were at opposite ends of the two strips. Each batch of infectors was of similar age when exposed and each was left in position for 3 weeks before the plants were uprooted and destroyed. Alternate plots in each strip were left without infectors. Eight infector units of eight whole progenies were harvested from each treated and untreated plot and grown on for inspection the following year.

In both 1957 and 1958, symptoms of primary leaf roll were noticed on many plants in the plots exposed to infection in late June and early July and the results obtained by growing on the tuber progenies (Table 5) confirm that leaf roll spread much more from infectors exposed in late June and early July than from those exposed later. The frequencies of infected plants and the proportions of such plants which produced totally infected progenies decreased with increasing distance from

the infectors and with increasing age of the Majestic crop. Very few plants became infected in the plots without infectors, and these were mostly in plots adjacent to those in which infectors were exposed early in the season. Although factors affecting the survival and dispersal of aphids from the infectors cannot be discounted and the virus may have been inefficiently transported to the tubers from haulms infected late in the season, the most plausible explanation of these results is that susceptibility of the Majestic plants to infection by leaf-roll virus declined sharply within 6–8 weeks after emergence.

TABLE 6. *Spread of leaf-roll virus in potato 'seed' crops in eastern Scotland*

	1955					1956				
County	Site no.	Variety	Date crop planted	% plants *LR* in sample	% plants infested *M. persicae* mid-July/ mid-Aug.	Site no.	Variety	Date crop planted	% plants *LR* in sample	% plants infected *M. persicae* mid-July/ mid-Aug.
Midlothian	1	**Epicure**	—	6·7	54/0	2	**Epicure**	5 March	16·7	100/0
	2	Kerr's Pink	—	100·0	60/0	5	Redskin	12 April	76·7	90/0
	3	Epicure	—	6·7	27/0					
	4	Redskin	—	100·0	100/0					
	5	Redskin	—	100·0	63/0					
Fife	28	**Kerr's Pink**	—	38·3	12/30	13	**Kerr's Pink**	12 April	30·0	60/93
	27	Golden Wonder	—	23·3	0/0	14	Redskin	—	11·7	33/75
						15	King Edward	—	11·7	48/93
						16	Majestic	—	15·0	27/84
						17	Redskin	25 March	25·0	51/87
Angus (south)	30	**Redskin**	—	58·3	24/96	28	**Redskin**	—	6·7	9/30
	32	**Redskin**	—	81·7	21/75	22	**Redskin**	14 April	46·7	21/75
	34	**Majestic**	7 May	1·6	0/24	19	**Majestic**	23 April	6·7	0/0
	35	**Redskin**	10 May	3·3	0/54	20	**Kerr's Pink**	23 April	5·0	0/3
	36	**Home Guard**	8 May	16·7	18/93	21	**Home Guard**	23 April	0·0	6/0
						24	Kerr's Pink	14 April	6·7	12/57
						25	Kerr's Pink	14 April	3·3	0/3
						26	Majestic	—	0·0	0/0
						27	Up-to-date	12 April	1·7	0/3
Angus (north)	49	**Kerr's Pink**	10 May	23·3	0/27	32	**Kerr's Pink**	—	0·0	0/3
	50	**Majestic**	12 May	37·0	0/72	31	**Majestic**	15 May	3·3	3/36
	51	**Bishop**	10 May	30·0	0/72	33	**Bishop**	12 May	0·0	3/9
	52	**Arran Pilot**	5 May	11·7	3/48	34	**Arran Pilot**	12 May	0·0	0/15
						29	Royal Kidney	22 April	0·0	0/3
						30	Kerr's Pink	14 April	8·3	0/21
Perth (south)	39	**Epicure**	13 May	2·0	3/18	39	**Epicure**	15 May	0·0	0/0
	40	**Majestic**	8 May	1·7	3/39	40	**Majestic**	20 May	0·0	0/9
	37	Majestic	—	1·7	0/15	35	Kerr's Pink	15 May	8·3	0/12
	38	Majestic	16 May	16·7	12/51	36	Kerr's Pink	—	3·3	6/45
						37	Majestic	7 May	0·0	0/12
						38	King Edward	4 May	1·7	3/6
						41	Arran Pilot	16 May	0·0	3/9
						42	Arran Consul	27 April	10·0	9/18
Perth (north)	42	**Majestic**	10 May	13·3	6/30	43	**Majestic**	7 May	0·0	0/6
	44	**Up-to-Date**	13 May	3·3	0/24	44	**Kerr's Pink**	12 May	0·0	0/6
	48	**Kerr's Pink**	23 May	5·0	0/24	48	**Kerr's Pink**	12 May	0·0	0/0
	43	**Arran Consul**	8 May	35·0	0/57	45	**Majestic**	14 May	0·0	0/3
	45	**Majestic**	15 May	3·3	3/27	46	**Majestic**	23 May	0·0	0/0
						47	King Edward	18 May	0·0	0/3

Spread of leaf roll in field crops

Ten leaf-roll infectors were exposed in each of twenty-five field crops in 1955 and in thirty-six such crops in 1956, and ten infector units of six plants were harvested from each. All the crops were being grown for 'seed' and they fall conveniently into six regional groups: north and south Perth, north and south Angus, Fife and Midlothian (Perth and Angus are arbitrarily divided by a line drawn from Dunkeld, in the west, to Arbroath, in the east). Table 6 shows the percentages of plants which produced leaf-roll-infected progenies sampled from each site (site numbers quoted from Figs. 1 and 2 of Fisken, 1959*b*), together with the percentages of plants infested by *M. persicae* in each crop in mid-July and mid-August (data from Fisken, 1957 and unpublished). These last are estimates based on aphid counts made monthly, in crops in Fife and Midlothian, or weekly or fortnightly in most crops in Angus and Perth (Fisken, 1959*b*).

In both years, leaf-roll virus spread in all districts except in north Perth in 1956. The amount of spread differed greatly between years, between areas and between crops within areas. Interpretation of these figures is difficult because of differences between varieties, both in susceptibility to infection by, and as sources of, leaf-roll virus. (Plants of varieties readily distinguishable from the indicator crops were always used as infectors.) Such differences may, for example, account for the unusually large amount of spread of leaf-roll virus in an isolated crop of Arran Consul in north Perth in 1955 (site 43, Table 6), a particularly susceptible variety (Chambers, unpublished). However, the same varieties were grown at fifteen of the sixteen sites sampled in both years (shown in heavy type in Table 6), and at fourteen of these, leaf roll spread more in 1955 than in 1956. In all the crops sampled, plants next to the infectors became infected more frequently than those more distant, and about two-thirds of the tuber progenies were only partially infected.

As relatively few crops in each area can be sampled in a survey of this kind, it would be wrong to suppose that those selected necessarily represent the whole area. However, although the amount of virus spread differed much between crops within areas, the figures suggest that leaf roll was more likely to spread in Midlothian, Fife and south Angus than in north Angus and Perthshire. The Midlothian crops were all near market gardens: those in Fife were coastal, as were the south Angus crops where virus spread most in 1955 (sites 30 and 32) and 1956 (site 22). Much spread was often associated with early colonization of crops by *M. persicae*, as indicated by the percentage of plants infested in mid-July. There is some indication that spread of virus was influenced by the rate at which aphid infestation built up in crops and was least in crops which became colonized late in the season.

Discussion

Our results suggest that, at Invergowrie, variations in the extent of spread of leaf roll and virus *Y* reflect aphid activity during the 4–6 weeks after potato plants emerge, when they are highly susceptible to infection. In 1954, 1955 and 1956,

years for which data on aphid infestation of the Invergowrie crops are available (Fisken, 1957, 1959*b*), most of the season's spread of virus *Y* and at least 25 % of that of leaf roll occurred in late June and before *Myzus persicae* was detected either by leaf counts or by trapping. Leaf roll consistently spread less and, in 1955 and 1957, rather later than virus *Y*. This is the reverse of the situation usually found in English ware-growing districts (Doncaster & Gregory, 1948). There is no evidence that either virus was introduced by aphids migrating into the crop and much to show that spread occurred from sources of infection within the crops.

In most parts of eastern Scotland, the summer dispersal of *M. persicae* from potato crops occurs late in the season and therefore at a time when potato plants resist infection by viruses. Consequently, the extent of virus spread may be expected to vary both with the time of arrival of the spring migrants and the rate at which crops become infested by *M. persicae*, factors which Fisken (1959*b*) found differed from year to year and from one district to another.

There are too many variables to attempt any detailed correlation between the spread of leaf roll in field crops and the aphid data collected by Fisken (1957, 1959*b* and unpublished). In general, however, the results confirm the conclusion already reached by Hollings (1955, 1957) that districts in which crops consistently become colonized by *M. persicae* late in the season are those in which viruses spread least. Together, Fisken's (1959*b*) and our results suggest that leaf-roll and *Y* viruses are more likely to spread in coastal than in inland districts of eastern Scotland. In 1945, leaf-roll virus spread rapidly in coastal districts. Symptoms of primary leaf roll were a feature of the 1945 epidemic and were also seen in 1955, a year when potato crops became colonized early in the season, and their appearance implies early spread of virus. Spring migrants seem rarely to spread leaf-roll virus over long distances. Hence the occasional epidemics seem to occur because *M. persicae* arrive early in the season, when plants are highly susceptible, and spread the virus from the few sources of infection within the crops. Although districts of eastern Scotland clearly differ considerably both from year to year and latitudinally in their suitability to produce high-grade seed, more detailed evidence is needed before the differences can be fully explained. The necessary evidence can only be provided by further extensive survey work and by intensive study of the relationships between aphid behaviour and virus spread.

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AN ATTEMPT TO INHIBIT THE MULTIPLICATION OF TOBACCO MOSAIC VIRUS IN TISSUE CULTURE BY ITS ANTISERUM

By K. A. MICZYNSKI*

Rothamsted Experimental Station, Harpenden, Herts

Extracts from tobacco tissue cultures infected with tobacco mosaic virus grown in medium containing antiserum to the virus had only one-half to one-sixteenth as much virus as extracts from tissues grown in medium without the antiserum. When tissues grown with antiserum were thoroughly washed before they were extracted, the extracts contained as much virus as extracts of tissues grown without antiserum. The antiserum did not affect virus multiplication, but antibodies in the tissues may have precipitated virus either in the cells or when the tissues were macerated.

It is generally thought that proteins do not move through the walls of cells but there are claims that they do. Kraus, Portheim & Yamanouchi (1907) and Moritz (1934) reported that animal proteins could enter roots and travel to the leaves where they could be detected serologically. Also Murphy & Syverton (1958) reported that mouse encephalomyelitis and poliomyelitis viruses entered the roots of plants growing in water culture and sometimes moved to the leaves. Brachet (1955) and Hamers-Casterman & Jeener (1957) found that ribonuclease could pass through the cell wall and alter the metabolism of the cells.

These reports prompted me to see whether the multiplication of tobacco mosaic virus (TMV) was inhibited in tissue cultures growing in media containing antiserum to TMV.

Methods

A tobacco tumorous callus tissue infected with TMV was supplied by Dr Kassanis and grown as described by him (Kassanis, 1957). Two antisera, titres 1/1280 and 1/2560, respectively, were prepared by injecting rabbits intravenously with a purified preparation of TMV. These were kept frozen because phenol as a preservative proved to be toxic to the tissue. The sera were sterilized by passing them through sintered glass filters of porosity 5. The sterile sera were mixed with autoclaved media before the agar set. There were usually three treatments each with twelve tubes: (1) control medium, consisting only of the basic medium; (2) the basic medium with 2·5 % (v/v) sterile TMV antiserum; (3) the basic medium with 2·5 % (v/v) sterile normal rabbit serum. The tissues were grown for 7–13 weeks before the virus concentration was estimated. The fresh weight of the tissues grown under the three treatments did not differ significantly. The concentration of virus

* This work was done while the author was on leave from the Botanical Institute, College of Agriculture, Cracow, Poland.

was estimated serologically, by finding the highest dilution at which a visible precipitation was formed when the clarified extract was titrated against antiserum at 1/100. The sap was clarified by heating for 10 min. at 60° C. and centrifuging. Infectivity tests made on *Nicotiana glutinosa* in some experiments gave results that agreed closely with those from the serological tests.

Experimental work

The serological titres of the extracts from tissues grown in the basic medium and those from tissue in medium with normal serum were always the same and always greater than the titre from the tissue grown in medium with antiserum. Of the five experiments summarized in Table 1, the decrease in titre in four depended on the length of time the tissues were kept in media containing antiserum. In Exp. 5 the virus titre was only halved although the tissues were treated for 11 weeks. The tissues were rinsed and dried on blotting paper before extracting the sap. However, when tissues were washed in running tap water for many hours the difference in titre between those treated with antiserum and untreated tissues was decreased

Table 1. *The effect of homologous antiserum on the serological titre of tobacco tumorous tissues infected with tobacco mosaic virus*

Exp.	Antiserum titre	Period culturing (weeks)	Ratio of virus titre of tissues grown in normal serum and antiserum				
			Without washing	After washing lasting for			
				24 hr.	48 hr.	72 hr.	6 days
1	2560	7	8	—	—	—	—
2	2560	11	16	4	—	—	—
3	1280	8	4	—	1	—	—
4	1280	13	16	8	—	2	—
5	1280	11	2	—	—	—	2

(Table 1). In Exp. 4 (Table 1), for example, the titres of the tissues grown in normal serum and in antiserum differed by sixteen times, but after 24 or 72 hr. washing the difference between the two tissues became eight and two times, respectively. The virus titre of untreated control tissues subjected to prolonged washing was twice that of the unwashed tissues.

Tissues dipped in sterile antiserum diluted 1/40 in saline for periods varying from 1 to 5 days also gave lower titres than untreated tissues. When the tissues were kept for 1 day in antiserum, then rinsed and the sap extracted, the titre in one experiment fell to 1/4 of that of the control, in another to 1/8 and after 5 days to 1/16. However, when the tissues were washed for 2 days in running tap water before the sap was extracted the titre of treated and control tissues was the same. These results show that although the titre of extracts is affected, the antiserum is obviously not inhibiting virus multiplication. The most likely explanation for the phenomenon is that antibodies either penetrate the intracellular spaces or are adsorbed on the cell walls. The amount of antiserum needed to remove the one-fifteenth to one-sixteenth

of virus was found by adding different volumes of antiserum (at 1 20) to extracts of untreated tissues before they were clarified. The volume needed was about 0·1 ml. of antiserum, which equals 5–10 % of the volume of the tissue. The tissues used are very firm with few obvious intracellular spaces, but the results suggest they accommodate a volume of antiserum equal to over 5 % of their total volume. To test the possibility that the antibodies are adsorbed on the outer cell walls, antiserum containing fluorescent antibodies was prepared by the method described by Nairn, Chadwick & McEntegart (1958). Tissues dipped for 2 days in this had areas which were strongly fluorescent even after washing, showing that some fluorescent protein was still adsorbed on the cell walls.

That the antibodies do not inhibit virus multiplication was further shown by an experiment in which the tissue was recultured four times in media containing TMV antiserum. At each reculture a piece about 1 10 to 1 20 of the volume of the parent tissue was transferred to new medium containing antiserum, where it was kept for 5–6 weeks. After 22 weeks the extracts of these tissues had a titre 1 8 that of tissues in medium with normal serum, so four serial exposures to antiserum did not affect the titre any more than did one exposure. The titre did not differ from that of the control when the tissues were washed for 4 days before making the extracts.

The experiments leave no doubt that the antiserum did not affect virus multiplication, but do not exclude the possibility that antibodies entered the cells. There seem to be two possible explanations for the difference in virus titres in extracts. One is that the antibodies penetrated the cells and precipitated some virus and so prevented its complete extraction until the antibodies were removed by washing; the other is that antibodies were adsorbed on cell walls or contained in the intracellular spaces and precipitated some virus when the tissues were macerated.

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RED CLOVER MOTTLE VIRUS

By R. C. SINHA*

Rothamsted Experimental Station, Harpenden, Herts

(With Plate 11)

A virus, provisionally named red clover mottle virus (RCMV), isolated from red clover plants in England, seems distinct from any previously described. It was transmitted by mechanical inoculation of sap to many legumes and to *Gomphrena globosa* L., but it was not transmitted by six aphid species, or through soil or through seeds.

RCMV is inactivated in 10 min. between 60 and 63° C., and in 8 days at 18° C., but survives for long periods at −20°; sap was not infective when diluted more than 1/1000. The virus is soluble in the pH range (4–7) in which it is stable. It was precipitated without inactivation by 50% saturated ammonium sulphate solution, but it was inactivated by ethanol or acetone. Partially purified preparations contained polygonal particles about 28 mμ in diameter. Serological tests showed no antigens in common with broad bean mottle, true broad bean mosaic or lucerne mosaic viruses.

In 1957 Dr T. W. Tinsley found diseased red clover plants on Rothamsted farm from which he transmitted a virus to red clover seedlings growing under glass. Preliminary observations suggested that this virus differed from any previously described, and this was confirmed by the studies on its properties and host range, described below. The virus will be called red clover mottle virus (RCMV).

Materials and methods

Stock cultures of RCMV were maintained in red clover plants (*Trifolium pratense* L.) but these take 12 days or more to develop symptoms and yield little sap, so the virus was propagated for quantitative work in the primary leaves of French bean (*Phaseolus vulgaris* L. var. Prince), which produce necrotic local lesions 3–4 days after inoculation; these leaves were also used to assay the infectivity of virus preparations.

Inoculations were made by gently rubbing the upper surface of leaves with the forefinger wet with inoculum. When 'Celite' (a diatomaceous earth) was used to increase the number of lesions, it was mixed with the inoculum. Infectivity assays were made by counting local lesions: when several inocula were compared they were distributed among half-leaves so that each occurred equally often on right and left halves and so that each possible combination of inocula on a leaf occurred equally often. Each inoculum was inoculated to at least eight half-leaves.

* Present address: Botany Department, University of Illinois, Urbana, Ill., U.S.A.

Results

Host range and symptomatology

Trifolium pratense L. (red clover). The first visible symptom in red clover seedlings inoculated under glass appeared 12–15 days after inoculation, when the areas along the veins of the youngest leaves became pale. This vein-clearing was temporary and was succeeded by general chlorosis, mottling, chlorotic rings and spots (Pl. 11, fig. 1), with leaves sometimes crinkling. Plants naturally infected in the field show similar symptoms. The symptoms are of the same type as those caused by red clover vein mosaic virus (Osborn, 1937).

Trifolium incarnatum L. (crimson clover). The young leaves show vein-clearing and chlorosis, which usually starts from the base and extends upward, producing dark green streaks in the chlorotic leaves (Pl. 11, fig. 2).

Trifolium repens L. (white clover) and *Melilotus alba* Desr. (sweet clover). Both species show vein-clearing in the young leaves. Later, in sweet clover chlorotic spots develop on new leaves but there is no general mottling or distortion as described for white clover mosaic (Pierce, 1935).

Phaseolus vulgaris L. var. Prince (French bean). The usual reaction is to form red necrotic local lesions 3–4 days after inoculation (Pl. 11, fig. 3), but in summer the lesions sometimes consist of a chlorotic halo with a necrotic centre. Similar necrotic local lesions also form in the variety Comtesse de Chambord. Usually symptoms are confined to inoculated leaves, but chlorotic spots, from which the virus was isolated, occasionally appeared in the uninoculated trifoliate leaves. The local lesions resemble those caused by lucerne mosaic virus, which also usually remains restricted to the inoculated leaves. Lucerne mosaic virus, however, is readily distinguished by its ability to infect many non-leguminous species (Price, 1940).

Vicia faba L. (broad bean). A few brown necrotic local lesions develop 6–10 days after inoculation, and are followed by systemic symptoms which show by the death, first of the stem apex, and later of the plant. The youngest leaves sometimes show a faint mottle or chlorotic spotting before the plant collapses. Stems sometimes show black streaks (Pl. 11, fig. 4). In total the symptoms resemble those caused by broad bean mottle virus (Bawden, Chaudhuri & Kassanis, 1951).

Soja max Piper (soybean). When seedlings are inoculated on their primary leaves, the first symptoms appear 10–12 days later as a faint mottling or large irregular chlorotic blotches on the trifoliate leaves. The third and successive trifoliate leaves show dark green or yellow puckered areas. The leaves become thick and chlorotic and fall prematurely. Pods produced by diseased plants look normal. Soybean mosaic virus causes similar symptoms (Conover, 1948) but is restricted to soybean.

Pisum sativum L. (pea). Five varieties (Gradus, Little Marvel, Alderman, Onward and Canadian Wonder) all became systemically infected when inoculated with RCMV. They showed vein-clearing of the young systemically infected leaves, followed first by vein-banding and then by a general chlorosis. Leaves produced

after infection were small and distorted, often showing small dark green blotches (Pl. 11, fig. 5)—symptoms resembling those caused by pea mosaic and bean yellow mosaic viruses (Pierce, 1935; Zaumeyer, 1940; Zaumeyer & Wade, 1935, 1936).

Vigna sinensis Endl. (cow pea). The only symptoms were necrotic local lesions, resembling those caused by lucerne mosaic virus (Milbrath & McWhorter, 1954).

Gomphrena globosa L. Symptoms showed only in inoculated leaves as reddening of the veins and red interveinal spots, somewhat resembling those produced by red clover vein mosaic virus (Ramamurthi, Ross & Roberts, 1958).

Attempts to find other non-leguminous hosts failed. The following species were inoculated, but developed no symptoms and no virus was isolated from them: *Nicotiana tabacum* L. var. White Burley, *N. glutinosa* L., *Chenopodium amaranticolor* Coste and Reyn., *Datura stramonium* L., *Lycopersicum esculentum* Mill., *Cucumis sativus* L. and *Brassica rapa* L.

Transmission

Diseased plants were distributed in the field at random, giving no indication of either the initial source of the virus or of its mode of spread.

RCMV was not transmitted by any of the following six species of aphids: *Acyrthosiphon pisum* (Harris), *Myzus persicae* (Sulzer), *M.* (*Sciamyzus*) *cymbalariae* Stroyan, *Megoura viciae* (Buckt.), *Aphis fabae* Scop., *Aulocorthum circumflexum* (Buckt.). In some tests the aphids fed undisturbed on infected red clover leaves for up to 2 days before they were transferred to healthy seedlings, on which they were then left for 2–4 days. In other tests the aphids were first starved for 4–6 hr., then left 2–5 min. on the infected leaves before they were placed on healthy seedlings. More than 1000 aphids of each species were used in these tests. Red clover vein mosaic, lucerne mosaic and bean yellow mosaic viruses are each transmitted by one or more of these aphid species (see Smith, 1957).

Tests for seed transmission were made with soybean, because infected red clover plants, the only known natural host of RCMV, set very few seeds. None out of 164 soybean seedlings raised from seed set by infected plants showed symptoms. Lack of seed transmission of RCMV in soybean does not, of course, exclude the possibility that it may be seed-transmitted in other hosts (Bennett, 1944).

Tests for soil transmission were made by sowing 100 pea seedlings in soil collected beneath diseased red clover plants in the field. Six weeks after sowing, sap was extracted from the roots and shoots of the pea plants and inoculated to French bean, none of which showed symptoms.

Properties in vitro

Thermal inactivation point. When 2 ml. samples of infective French-bean sap were heated for 10 min. at various temperatures infectivity was destroyed at 63° C. Much infectivity was lost at temperatures considerably below the thermal inactivation point, some even at 45° C. (Table 1).

Longevity in vitro. The infectivity of sap falls considerably during 3 days at 18° C. and is lost after 6 days. Partially purified preparations were infective after

6 days but not after 8 days at 18° C., showing that the stability of the virus was only slightly enhanced by removing many of the components of crude sap.

Effect of freezing. Infective sap kept at −20° C. for several months was as infective as fresh sap. Sap kept at −20° C. for 5 days was more infective than a sample kept for 5 days at 4° C.

TABLE 1. *Thermal inactivation of red clover mottle virus*

Dilution of sap*	Unheated	French-bean sap heated for 10 min. at (° C.) 45	50	55	63
1/1	1652†	1490	1041	201	0
1/10	330	229	20	0	0
1/100	19	5	0	0	0

* Sap diluted after heating.

† Total number of lesions on eight half-leaves of French bean, 'Celite' was mixed with the inoculum.

TABLE 2. *Effect of dilution on infectivity of previously frozen French-bean sap*

Dilution of sap	Exp. 1 With Celite	Exp. 1 Without Celite	Exp. 2 With Celite	Exp. 2 Without Celite
1/2	2080*	402	1600	363
1/10	301	13	44	15
1/50	11	0	5	1
1/250	3	0	1	0

* Total number of lesions on eight half-leaves of French bean.

Dilution end-point. When sap from infected French-bean leaves was diluted serially in distilled water, mixed with 'Celite' and inoculated to French-bean leaves, infections were usually obtained at 1/1000 but not at 1/10,000. Frozen and thawed sap, diluted 1/10 often produced few or no lesions, although the undiluted sap produced many. This sudden decrease in infectivity of frozen sap on dilution was usual (Table 2) and sometimes also happened with fresh sap. Adding 'Celite' to the inoculum sometimes prevented the sudden decrease.

Diluting thawed sap in sap freshly extracted from healthy French-bean leaves, or adding sucrose to the sap before freezing (Kassanis & Slykhuis, 1959), did not prevent the loss of infectivity caused by dilution.

Precipitation of RCMV by various agents. After clarifying by freezing, thawing and centrifuging at low speed, sap was mixed with various amounts of acetone, ethanol or a saturated solution of ammonium sulphate. The sediments obtained when the mixtures were centrifuged at low speed were resuspended in a volume of distilled water equal to that of the original sap, centrifuged and the infectivity of the supernatant fluids tested. Most of the infectivity was recovered from precipitates produced in solutions 50% saturated with ammonium sulphate (Table 3), but high concentrations inactivated and little virus was recovered from precipitates produced by acetone or ethanol.

Effect of pH *value.* Clarified infective sap was adjusted to a range of pH values by adding M/100 hydrochloric acid. The preparations were then centrifuged at low speed, the supernatant fluids poured off and sediments resuspended in distilled water. The pH value of the supernatant fluids and resuspended sediments was adjusted to pH 7, and infectivity of all the samples assayed. At pH 6 and 7 the infectivity of supernatant fluids was very similar to that of untreated sap and the sediments contained little virus. Between pH 6 and 4 infectivity gradually decreased with decrease of pH value but the infectivity that remained at pH 4 was all in the supernatant fluid (Table 4). RCMV seems to be soluble in the pH range below 7 in which it is stable and therefore differs from lucerne mosaic virus which precipitates in acid solutions and is least soluble at pH 4·5 (Ross, 1941).

TABLE 3. *Precipitation of red clover mottle virus by ammonium sulphate solution*

Percentage saturated solution	Infectivity of resuspended precipitate		
	Exp. 1 (1/5*)	Exp. 2 (1/10)	Exp. 3 (1/10)
10	0†	—	—
20	0	0	8
30	86	0	95
40	496	235	680
50	809	907	945
60	—	142	—
70	—	—	65
Untreated sap	588	1263	1352

* Dilution of samples before inoculation.
† Total number of lesions on eight half-leaves of French bean.
— Treatment not included.

TABLE 4. *Effect of* pH *value on infectivity and solubility of red clover mottle virus in French-bean sap**

pH value	Exp. 1		Exp. 2	
	Supernatant fluid	Sediment	Supernatant fluid	Sediment
3	0†	0	41	0
4	532	0	735	0
5	714	12	742	112
6	1113	30	910	49
7	882	156	1182	385

* The solutions, adjusted to the appropriate pH value by adding M/100 hydrochloric acid, were centrifuged at low speed and the sediment resuspended in the original volume: all samples were adjusted to pH 7 before inoculation.
† Total number of lesions on eight half-leaves of French bean.

Purification and electron microscopy

RCMV was partially purified by a method similar to that used by Steere (1956) for tobacco ringspot virus. A 1:1 mixture of *n*-butanol and chloroform was mixed with an equal volume of clarified sap, shaken for 5 min. and then centrifuged for 10 min. at 10,000 r.p.m. Of the three resulting layers the uppermost was poured off and left at 18° C. for 12–16 hr., after which insoluble material was removed by centrifuging for 5 min. at 10,000 r.p.m. The supernatant fluid was centrifuged for 2 hr. at 25,000 r.p.m. (75,000 *g*), the pellet resuspended in M/90 phosphate buffer (pH 7) and centrifuged for 2 min. at 10,000 r.p.m.; the supernatant fluid was then given a further cycle of differential centrifugation. The final preparation was resuspended in phosphate buffer using 1/10 the initial volume. Electron micrographs of such preparations showed particles of about 28 mμ in diameter, several of which appear polygonal rather than spherical (Pl. 11, fig. 6). No such particles were found in preparations from sap of healthy plants subjected to the same procedures. The purified preparations were highly infective and serological tests indicated that more than half the virus in the crude sap was present in the final preparations.

Serological reactions

Antiserum that reacted specifically with sap from French-bean plants infected with RCMV was prepared by injecting a rabbit with preparations partially purified by the method already described. Six injections, each of 2 ml., were given at 3-day intervals and the rabbit bled 10 days after the last injection. The antiserum was stored at 4° C. with thiomersalate (1/5000) added to prevent bacterial growth. Precipitation tests were made by mixing 1 ml. of dilute antigen and 1 ml. of dilute antiserum in tubes of 8 mm. diameter, which were incubated in a water bath at 37° C. When the antiserum was titrated against clarified sap at a constant dilution (1/4), it gave a precipitation end-point of 1/160 with sap from infected French bean and 1/20 with sap from uninfected French bean. When infective sap was titrated against the antiserum diluted 1/50, it gave a precipitation end-point of 1/80. The precipitate was granular and compact, typical of those caused by isometric particles.

RCMV did not precipitate with antiserum prepared against lucerne mosaic virus, broad bean mottle virus and true broad bean mosaic virus (Quantz, 1953) and these viruses did not precipitate with antiserum to RCMV, either in tube or gel-diffusion tests (see Kleczkowski, 1957).

RCMV obviously differs from red clover vein mosaic, white clover mosaic, bean yellow mosaic and pea mosaic viruses, for these all have filamentous particles (see Brandes & Wetter, 1959). The serological tests and its properties also separate it from the several legume viruses which have isometric particles.

The prevalence and economic importance of RCMV in field conditions are uncertain.

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EXPLANATION OF PLATE 11

Fig. 1. Red clover (*Trifolium pratense*) leaf systemically infected with red clover mottle virus, showing mottling, chlorotic rings and spots.

Fig. 2. Crimson clover (*Trifolium incarnatum*) leaf systemically infected with red clover mottle virus, showing dark green streaks in the otherwise chlorotic leaves.

Fig. 3. French bean (*Phaseolus vulgaris*, var. Prince) leaf showing necrotic local lesions, 4 days after inoculation with red clover mottle virus.

Fig. 4. Broad bean (*Vicia faba*) shoot systemically infected with red clover mottle virus, showing top necrosis.

Fig. 5. Pea (*Pisum sativum*, var. Onward) leaf systemically infected with red clover mottle virus showing distortion and chlorotic spots.

Fig. 6. Electron micrograph showing particles about 28 mμ in diameter from a purified preparation of red clover mottle virus. Note that some particles have a polygonal outline.

Photographs by Mr F. Cowland; electron micrograph by Mr H. L. Nixon and Mr R. D. Woods.

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SINHA—*Red clover mottle virus*

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SOME EFFECTS OF TEMPERATURE AND OF VIRUS INHIBITORS ON INFECTION OF FRENCH-BEAN LEAVES BY RED CLOVER MOTTLE VIRUS

By R. C. SINHA*

Rothamsted Experimental Station, Harpenden, Herts

Keeping French-bean plants before inoculation at 36, 32 or 28° C. for 1–2 days increased their susceptibility to infection with red clover mottle virus, but longer exposures to 36 and 32° C. decreased susceptibility. Susceptibility increased most rapidly at 36° C. The number of infections was unaffected by changes in post-inoculation temperatures between 12 and 24° C., but decreased above 24° C. The rate virus multiplied increased with increase of temperature up to 28° C., but the maximum virus concentrations reached at 18, 24 and 28° C. were very similar and above the maximum reached at 30° C.

Thiouracil inhibited infection slightly but neither it nor azaguanine affected the multiplication of red clover mottle virus in French bean. Trichothecin inhibited infection and interfered with virus accumulation. Inhibition of infection was associated with macroscopic injury to the leaves, and washing leaves up to 1 hr. after inoculation prevented both inhibition and leaf damage. Virus multiplication was not resumed when leaves were transferred from trichothecin solutions to water.

Introduction

Of the many environmental factors that affect the initiation and development of virus infection in plants, temperature seems the most important (Kassanis, 1957). Most plants show increased susceptibility to infection by all mechanically transmitted viruses when they are kept at high temperatures (36° C.) before they are inoculated, but different viruses show contrasting types of behaviour when plants are kept at 36° C. after inoculation. Some, like tobacco mosaic virus, infect readily in plants kept at 36° C., whereas others, like tobacco necrosis virus, do not, and inoculations that would produce many lesions in plants kept at 20° C. produce none in plants kept at 36° C. Different strains of one virus may behave in these contrasting ways; for example, many strains of cucumber mosaic virus do not multiply in plants kept at 36° C. (Kassanis, 1952) whereas one does (Hitchborn, 1956). The experiments described below were made to see in which category the newly described red clover mottle virus falls. Also described are the effects of substances previously found to inhibit infection and multiplication of some other viruses, and whose action seems to be influenced by the identity of the host plant in addition to that of the virus (Bawden & Kassanis, 1954; Badami, 1959).

* Present address: Botany Department, University of Illinois, Urbana, Ill., U.S.A.

MATERIALS AND METHODS

Red clover mottle virus (RCMV) was propagated, and infectivity assays were made, in the primary leaves of French bean (*Phaseolus vulgaris* L. var. Prince) (Sinha, 1960). In experiments on virus multiplication, undiluted infective sap, with 'Celite' added to increase the number of infections, was used as the original inoculum. After inoculation the leaves were thoroughly rinsed in a stream of tap water. Samples consisting of eight, randomly selected, half-leaves were taken from such leaves at intervals. The eight half-leaves in a sample were macerated together and their sap stored at −20° C., where infectivity is maintained over long periods, until all the samples from an experiment were collected. The extracts were then thawed and suitably diluted before they were assayed. Constant temperatures were obtained in thermostatically controlled glass-sided incubators, which kept to within 1° C. of the stated value.

RESULTS

Effect of temperature on lesion number

The effect of high temperature on lesion number was studied by inoculating French-bean plants either before or after they were exposed at 36° C. Before and after such exposures the plants were in the fluctuating temperature of the glasshouse (average 20° C.). Table 1 shows that pre-inoculation treatment at 36° C. for 24 hr. increased the susceptibility of plants to infection, but keeping them for a further 24 hr. returned it to the level of the unheated plants. By contrast, few lesions developed in leaves of plants kept for 24 hr. after inoculation at 36° C. and none when plants were kept for 48 hr.

TABLE 1. *Effect on lesion number of exposing plants at* 36° C.

Exp. no.	Exposed before inoculation		Untreated	Exposed after inoculation	
	48 hr.	24 hr.		24 hr.	48 hr.
1	139*	235	141	18	0
2	177	250	174	71	0
3	172	297	197	17	0

* Average number of lesions per leaf for sixteen leaves.

Further experiments compared the effect of exposing plants before inoculation at 36° C. with effects at 28 or 32° C. Susceptibility increased most rapidly at 36° C., reached its maximum in 24 hr. and began to decrease after 40 hr. At 32° C. susceptibility increased less rapidly, reached a lower maximum at 24 hr. and started to decrease thereafter, but less rapidly than at 36° C. At 28° C., susceptibility increased slowly and was still increasing at 40 hr., the longest interval tested. The number of hours needed to give the maximum increase in susceptibility, therefore, decreases with increase of temperature and will probably also vary with the age of plants and with their physiological state at the time they are treated.

The results parallel those with other viruses (Kassanis, 1952), and resemble those with potato virus *Y* in *Physalis floridana* in that there is a length of exposure that increases susceptibility most (Nienhaus, 1957). They perhaps also indicate that the contradiction suggested by Lindner, Kirkpatrick & Weeks (1959) between the behaviour of tobacco mosaic virus in *Nicotiana glutinosa* and in cucumber cotyledons may be apparent rather than real. Kassanis (1952) found that pre-inoculation exposure at 36° C. increased susceptibility, whereas Lindner *et al.* (1959) found that pre-inoculation exposures at 32° C. made plants less susceptible than those kept at 20° C. The treatments, however, were not comparable, for whereas Kassanis kept his plants at high temperatures for at most 4 days, Lindner *et al.* raised their plants at the high temperature, an exposure which can be expected from my results to be long enough to decrease susceptibility instead of increasing it.

TABLE 2. *Effect of post-inoculation temperatures on lesion number*

(*a*) Temperatures below 24° C.

	Temperature (° C.)			
Exp. no.	12	16	20	24
1	125*	120	121	108
2	150	178	176	196
3	62	58	64	49
4	84	73	96	71
Average	105 (4+)	107 (3)	114 (2)	115 (2)

(*b*) Temperatures above 24° C.

	Temperature (° C.)		
Exp. no.	24	28	32
1	70*	53	3
2	38	21	2
3	36	19	0
4	108	74	0
Average	63 (2+)	42 (2)	1 (−)

* Average number of lesions per leaf for sixteen leaves.
\+ The number of days when lesions first appeared.
− Treatment not included.

The effect of high temperature after inoculation was also studied in more detail. Leaves kept at 36° C. for 10 hr. after inoculation, formed about half as many lesions as the control leaves, but very few lesions developed when the treatment was prolonged to 20 hr. Exposure at 32° C. had less effect; lesion number was halved after 20 hr. To find the optimum post-inoculation temperature for lesion formation by RCMV in French-bean leaves, plants were put immediately after inoculation at various temperatures and kept there until the lesions developed. Table 2 shows that the same number of lesions developed at 24°, 20°, 16° and 12° C. and that the number decreased above 24° C. The lesions took longer to appear at 12 and 16° C. than at 20–28° C. and even 6 days after inoculation were still small. The decrease

in number of lesions above 24° C. may happen because some virus from the inoculum becomes inactivated but it is also possible that the higher temperatures make some host cells unable to support virus multiplication.

Effect of temperature on virus accumulation

To study the accumulation of RCMV at various temperatures, the leaves were sampled at intervals of 1, 2, 4 and 6 days after inoculation. The infectivities of the samples (Table 3) show that the virus needed 4 days at 12° C. to reach a detectable level, 2 days at 18 or 30° C. and 1 day at 24 and 28° C. Virus accumulated most rapidly at 28° C.; not only was the infectivity of the sap more at 1 day at 28 than at 24° C., but the peak virus concentration was reached in 2 days, whereas 4 days were needed at 24 and 30° C., and 6 days at 18° C. The maximum virus concentrations reached at 18, 24 and 28° C. were very similar, and were greater than at 30° C. At 12° C. the virus concentration after 6 days was less than at the higher temperatures, but had the plants been left for longer periods, the peak concentration might have reached or perhaps exceeded that at other temperatures. Over the range 18–28° C. there was, however, no evidence that the maximum virus concentration increased

TABLE 3. *The accumulation of RCMV in inoculated leaves of French bean at different temperatures*

Exp. no.	Temp. (° C.)	Inoculum (reciprocal dilution)*	Days after inoculation: 1	2	4	6
			Total no. of lesions on eight half-leaves			
1	24	2	0	684	1845	1863
		10	0	85	322	320
	18	2	0	144	1322	1577
		10	0	10	355	406
	12	2	0	0	64	207
		10	0	0	0	28
2	24	2	0	212	865	1123
		10	0	26	137	158
	18	2	0	41	821	1294
		10	0	4	209	267
	12	2	0	0	51	435
		10	0	0	0	67
3	24	2	49	570	896	967
		10	0	77	268	298
	28	2	100	915	1090	1188
		10	7	58	131	103
4	24	2	5	421	962	951
		10	0	39	179	211
	28	2	31	1247	1348	1247
		10	0	336	294	350
	30	2	0	56	619	506
		10	—	—	—	—

* Reciprocal of dilution at which virus content of samples was assayed.
— Treatment not included.

with decrease of temperature, as happened with cucumber mosaic virus in tobacco leaves (Badami, 1959).

The fact that the virus eventually reaches similar concentrations at 18, 24 and 28° C. has two possible explanations. Either the initial rate of multiplication at 28° C. may not be maintained, or virus synthesis may become balanced at a certain virus concentration after different times at different temperatures. Harrison (1956) showed that a tobacco necrosis virus both multiplies and is inactivated in French-bean leaves at 30° C.: he suggested that inactivation also occurs at lower temperatures, and that the extent to which the virus accumulates at any temperature represents a balance between synthesis and inactivation. If this is so, inactivation of RCMV becomes important only above 28° C. Thus the difference between the virus content at 28 and 30° C. may reflect slower synthesis at 30° C., more rapid inactivation, or both. It is interesting to note that the optimum temperature for accumulation is higher than the optimum temperature for lesion formation, suggesting that the initiation and the progress of virus multiplication depend on different processes.

In general, the accumulation of RCMV in French-bean leaves follows the same pattern as that of the Rothamsted tobacco necrosis virus (Harrison, 1956), but there are two important differences. First, although tobacco necrosis virus has a con-

TABLE 4. *Effect on virus content of exposing virus-containing leaves at* 36° C.

Days at 36° C.	Infectivity of sap	
	1/2+	1/10
0	1715*	355
2	1181	66
4	85	5
8	7	0

* Total number of lesions on eight half-leaves.
\+ Dilution of samples tested.

siderably higher thermal inactivation point *in vitro* than RCMV, RCMV accumulates optimally at 28° C. and tobacco necrosis virus at 22° C. Secondly, the maximum virus content in French-bean leaves is reached sooner with RCMV than with tobacco necrosis virus, presumably because tobacco necrosis virus lesions spread along the leaf veins, whereas RCMV lesions are more strictly localized. Several viruses produce different kinds of lesions in plants kept at different temperatures and some that cause small discrete lesions at 20° C. cause larger lesions or chlorotic ones at high temperatures. No such temperature effects were noted with RCMV, which formed the same type of lesions at all temperatures.

Virus inactivation in French-bean leaves at 36° C.

RCMV clearly belongs to the group of viruses which does not multiply in newly infected plants kept at 36° C. All other viruses with this behaviour that have been tested are also inactivated when fully infected leaves are kept at 36° C., and whole

plants can be freed from infection by heat (Kassanis, 1957). I have not attempted to free systemically infected red clover plants from RCMV, but this could probably be done. Table 4 shows that the virus content of French-bean leaves kept at 20° C. until lesions developed fell after 2 days at 36° C. and little remained after 8 days.

Effect of inhibitors of virus multiplication

The effects of three virus inhibitors, thiouracil, 8-azaguanine and trichothecin were tested with RCMV in French bean. When mixed in the inoculum, thiouracil only slightly affected the number of infections. At 750 mg./l. it decreased lesion number to a quarter, and even at 1500 mg./l. a few lesions still developed. In this respect RCMV behaves like the Rothamsted tobacco necrosis virus in French bean (Bawden & Kassanis, 1954). Trichothecin (Bawden & Freeman, 1952) inhibited infection much more. The presence of only 50 mg./l. trichothecin in the inoculum decreased the numbers of infections and at 200 mg./l. it decreased them by 90%. Leaves in which trichothecin affected lesion number were always damaged. The effect on lesion number and damage to the leaves could be prevented, even when the inoculum contained 200 mg./l. trichothecin, by washing the inoculated leaves with water within 1 hr. of inoculation, but not later. This suggests that trichothecin passes into the leaves within an hour or so and then can no longer be removed by washing. The correlation of inhibition of infection with leaf damage supports earlier views that trichothecin acts primarily on the host (Bawden & Freeman, 1952; Gupta & Price, 1952). Exposing RCMV to trichothecin *in vitro* does not inactivate it.

TABLE 5. *Effect of thiouracil, azaguanine and trichothecin on virus multiplication*

		100 mg./l.		
Exp. no.	Water	Thiouracil	Azaguanine	Trichothecin
1	920*	914	909	10
2	476	492	500	24
3	256	202	268	14
4	220	247	226	0

* Total number of lesions on eight half-leaves. Detached leaves were floated 2 days after inoculation in the four fluids, and the virus content of the sap was assayed 7 days after floating.

To find the effects of inhibitors on virus multiplication, French-bean leaves were inoculated with RCMV, washed with running water and then kept in an incubator at 24° C. Two days after inoculation, when the lesions were already starting to appear, the leaves were detached and floated in water or in solutions containing 100 mg./l. thiouracil, azaguanine or trichothecin. All solutions also contained 0·3 g./l. sulphanilamide to prevent bacterial growth. The relative virus contents of the leaves were assayed 7 days after floating them in the solutions. The leaves were thoroughly washed and dried before their sap was extracted and its infectivity tested. Thiouracil and azaguanine had no effect on the virus content, but trichothecin greatly decreased it (Table 5). The leaves floated in trichothecin were again

damaged but less severely than when trichothecin was mixed with the inoculum. Transferring the leaves to water after 2 days in trichothecin solution did not restart virus multiplication. The total number of lesions produced in the leaves floated in the different fluids were very similar, but the lesions on those in the trichothecin solution were smaller than the others.

It seems likely that trichothecin acts by preventing further virus multiplication and did not inactivate virus already formed. The three inhibitors affect infection by RCMV in much the same way as they affect infection by tobacco necrosis virus in French bean. Possibly different results would have been obtained had another host been used, for Bawden & Freeman (1952) found that trichothecin inhibited infection more in beans than in *Nicotiana glutinosa*, and Bawden & Kassanis (1954) found that thiouracil affected the multiplication of the Rothamsted tobacco necrosis virus in tobacco but not in French bean.

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THE EFFECT OF CERTAIN PREDATORS ON THE NUMBERS OF CABBAGE ROOT FLY (*ERIOISCHIA BRASSICAE* (BOUCHÉ)) AND ON THE SUBSEQUENT DAMAGE CAUSED BY THE PEST

By D. W. WRIGHT, R. D. HUGHES* and J. WORRALL

National Vegetable Research Station, Wellesbourne, Warwick

(With Plate 12 and 4 Text-figures)

To determine the importance of beetle predators on the natural control of cabbage root fly, experiments were carried out in 1958 and 1959 using various types of barriers to obtain different levels of beetle populations on cauliflower plots. A barrier of DDT-treated straw, placed in the soil around some plots, decreased the numbers of beetles within them and allowed a greater number of eggs and larvae of cabbage root fly to survive than on the untreated plots, resulting in a greater crop damage. Another type of barrier allowed the beetles to enter plots but made it difficult for them to leave. On these, fewer cabbage root-fly eggs and larvae survived and the crop damage was much less than on the plots surrounded by straw barriers. Where plants were treated with insecticide the root-fly population was reduced to a minimum and crop yields were considerably increased. The insecticide, however, caused a reduction in the numbers of predatory beetles.

It has been shown in field experiments at Wellesbourne (Hughes, 1959; Hughes & Salter, 1959) that several species of beetle feed on cabbage root-fly eggs, and may consume more than 90% of all those laid on a brassica crop. This reduction in numbers is insufficient to prevent considerable crop loss; and chemical control of the remainder is necessary where valuable brassica crops are grown. The estimates of egg losses were based on the numbers of eggs recovered after different time intervals (Hughes, 1959). Mitchell (1959) observed *Bembidion lampros* (Herbst.) and *Trechus quadristriatus* (Schrank.) feeding on cabbage root-fly eggs in the field, and partially eaten eggs have been found there identical to those obtained from laboratory feeding experiments with these beetles (Hughes, 1959).

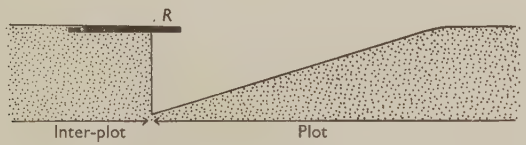
To demonstrate the effect of the predator complex on populations of the cabbage root fly, field experiments were carried out at Wellesbourne during April, May and June in 1958 and 1959 on early cauliflowers which were attacked by the first generation of the cabbage root fly. The wingless form of *Bembidion lampros* was the most abundant predator in the field over this period, moving into the plots in late March and April from overwintering sites in surrounding hedgerows and grass. In both years attempts were made to obtain different levels of beetle populations within the experimental plots by using barriers to restrict inward and outward beetle movement.

* Now with Entomology Division C.S.I.R.O., Canberra, Australia.

In 1958 barriers of plaited straw rope were used to deter the entrance of flightless beetles: in another treatment, similar straw barriers were used, but these were sprayed at intervals with DDT to increase their effectiveness. In 1959 straw barriers treated with DDT were again used, but instead of the untreated straw barrier, a one-way catch barrier was devised which allowed the beetles to enter the plots but restricted their outward movement, thus increasing predator density. In both years, plots of cauliflowers were included which received the standard insecticide treatment for the control of cabbage root-fly larvae, applied to the soil around the plant stem (Wright, 1956). In addition a control treatment was included in both years. These treatments provided crop-yield data which gave a measure of the damage caused by the pest, and therefore reflected the effects of different numbers of predators on them.

Experimental design, methods and records

The experiments in 1958 and 1959 were carried out in the same field, and the size, number of plots and crop cultivation, was similar in both years. The cauliflowers were transplanted in early April and harvested in June. Each plot contained 100 plants and was approximately square, measuring 111 sq. yd. To minimize the effects of treatments on the beetle populations of adjoining plots, 9-ft.-wide strips of



Text-fig. 1. Cross-section through catch barrier showing roofing felt (*R*) in position around the plot.

cultivated but uncropped land separated them. The four treatments used were replicated three times in a randomized block layout, and, for 1958, the treatments were as follows:

(*a*) Untreated. No barriers or insecticides used.

(*b*) Dieldrin plant treatment. The soil immediately around each plant was given an 80 c.c. drench of 0·03 % dieldrin emulsion in water.

(*c*) Straw barrier. A barrier of plaited straw rope approximately 4 in. diameter was pegged into position around the plot at a distance of $2\frac{1}{2}$ ft. from the outside row of plants. In addition two straw ropes placed at right angles across the plot divided it into four equal subplots.

(*d*) Straw barrier + DDT. Straw ropes, arranged as in (*c*), were sprayed at 10-day intervals with a 0·5 % DDT emulsion.

In 1959 treatments (*a*) and (*b*) were repeated, and treatment (*d*) was replaced by a straw-filled trench 6 in. wide and 6 in. deep. Treatment (*c*) was replaced by a catch-barrier treatment. This surrounded the plot $2\frac{1}{2}$ ft. from the outside row of

cauliflowers, and was made by removing soil at the edges of the plot so that it sloped down to a 3-in. vertical wall of soil enclosing the plot. A 4-in.-wide strip of roofing felt was placed flat on the soil, so as to overlap the wall from outside by approximately 1 in. (Text-fig. 1). Preliminary tests showed that the beetles moved readily on to, and fell over, the overlapping edge of the felt, into the plot area. Few beetles were able to climb out of the plot but many probably escaped through deep cracks in the soil which appeared during the dry weather of May and June.

Results of the 1958 experiment

In early June, many cauliflower plants showed wilting and stunting: the symptoms of severe cabbage root-fly attack. The number of affected plants rapidly increased, and many died as their root systems were destroyed by the larvae (Table 1). When the crop was mature the yield and quality of the crop from the various plots was also recorded (Table 1).

Table 1. *Yield of cauliflowers and numbers of plants severely affected in mid-June by cabbage root fly (75 plants/plot), Wellesbourne, 1958*

Treatment	Mean weight marketable heads (kg.)	Mean no. of 1st grade heads (> 5 in. diam.)	Mean no. of plants dead or severely stunted
(*a*) Untreated	57·2	43·3	14·3
(*b*) Dieldrin plant treatment	94·4	66·0	1·6
(*c*) Straw barrier	41·6	30·3	26·3
(*d*) Straw barrier + DDT	45·3	34·3	27·0
	S.E. = ±16·54, L.S.D. ($P = 0.05$ %) = ±40·5, $b > c, d$	S.E. = ±12·95, L.S.D. ($P = 0.2$ %) = ±19·4	S.E. = ±10·51, L.S.D. ($P = 0.2$ %) = ±15·8

There was a gradient in root-fly attack across the experimental area, and the three plots of each treatment occurred at different positions in the gradient, and therefore showed wide variations in attack. The yield data were similarly affected so that few of the differences obtained in the yield, quality, and in the numbers of plants killed or severely stunted were statistically significant. The data given in Table 1 indicate that the crops on treatments (*c*) and (*d*) were more heavily damaged than those on the untreated plots (*a*). Where the plants were treated with insecticide (*b*), the crop yield was increased by 65 %, as compared with the untreated plots, and there was a corresponding improvement in crop quality. Some of the differences between treatments can be seen from the photographs in Pl. 12.

Open glass jars were set just below the level of the soil surface on the plots of treatments (*a*) and (*d*), and the beetles caught in these traps during four separate periods, during late April and May, were recorded. On all occasions fewer beetles (chiefly *Bembidion lampros*) were trapped on the straw-barrier DDT plots than on the untreated plots; the total number caught on the former being only 28 % of that trapped on the untreated plots. Two egg counts made in May showed that there were approximately 50 % more eggs present on the straw-barrier DDT plots than on the untreated plots.

These results were consistent with the hypothesis that the straw barriers reduce the predator density within these plots, allowing more root-fly eggs to survive, thus producing an increase in damage on the crop.

Results of the 1959 experiment

The 1959 experiment was carried out on a site adjoining that used in the previous year. The plots were arranged in randomized blocks which were set at right angles to the gradient of root-fly attack observed in the previous year. Although a gradient of attack again occurred, it was much less marked than in the previous year, and this, combined with the altered plot arrangement, permitted a more accurate assessment of the performance of the various treatments. The numbers of plants severely affected by root fly and the yield data obtained are shown in Table 2.

Table 2. *Yield of cauliflowers and numbers of plants severely affected by cabbage root fly* (*75 plants/plot*), *Wellesbourne*, 1959

Treatment	Mean weight marketable heads (kg.)	Mean no. of 1st grade heads (> 5 in. diam.)	Mean no. of plants dead or severely stunted
(*a*) Untreated	27·9	12·0	17·3
(*b*) Dieldrin plant treatment	50·2	31·0	1·6
(*c*) Catch barrier	28·6	13·7	11·0
(*d*) Straw barrier + DDT	17·4	7·3	29·3
	S.E. = ± 6·58, L.S.D. ($P = 0{\cdot}05$ %) = ± 12·1, $b > a, c, d$	S.E. = ± 5·73, L.S.D. ($P = 0{\cdot}05$ %) = ± 10·5, $b > a, c, d$	S.E. = ± 6·33, L.S.D. ($P = 0{\cdot}05$ %) = ± 11·6, $d > a, b, c$, $a > b$

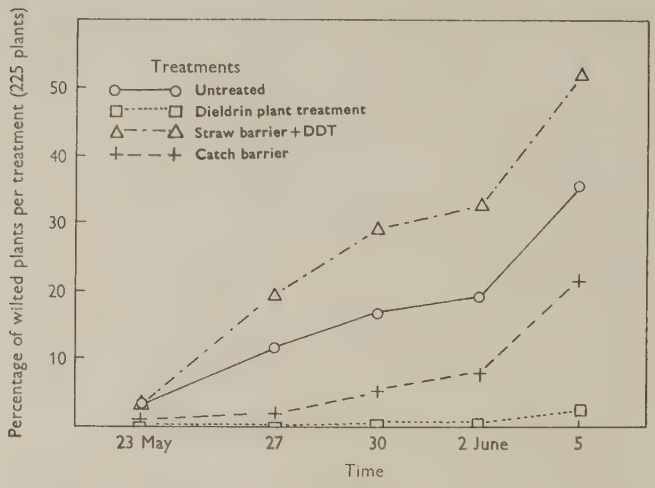
All standard errors are for differences of two means and based on 6 D.F.

Because of drought the crop yield was smaller than in 1958, but the same order of differences between treatments was recorded. The straw-barrier DDT treatment again showed the highest number of plants affected and the lowest yield. The catch-barrier treatment resulted in a slightly better performance than on the untreated plots.

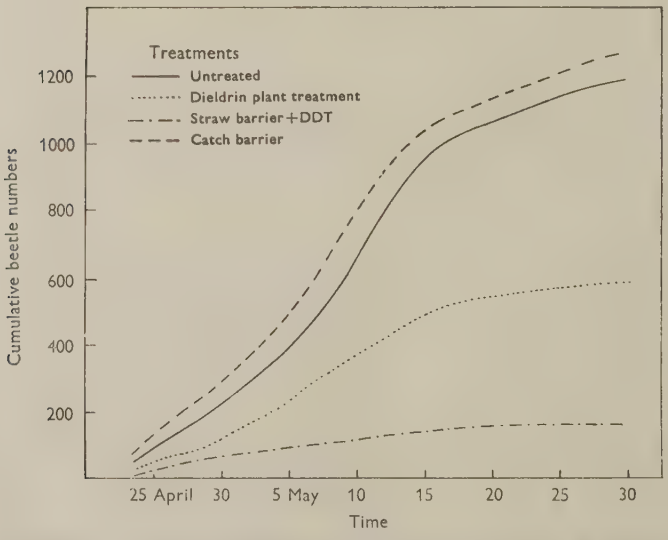
In addition to causing death or severe stunting of the plants the root fly may, at lower levels of infestation, cause temporary daytime wilting, particularly in dry soil. Wilted plants were first recorded on 23 May and their numbers rose steadily until early June, then more rapidly. The percentage of plants affected on the various treatments during the 2-week period before harvesting is shown in Text-fig. 2. Large and consistent differences occurred in the numbers wilted on the various treatments. The numbers affected on the straw-barrier plots were, on both occasions in June, significantly greater than the numbers on both the catch-barrier and dieldrin plant treatment plots, but the numbers of wilted plants on the straw-barrier plots were not significantly different from those on the controls.

From 21 April, when eggs of the root fly were first found on these plots, until the end of May, records were kept of the beetle and egg populations on all the plots.

Nine pit traps were set in each plot and emptied daily; the beetles were counted and then returned to the plot from which they were trapped. Egg counts (Hughes & Salter, 1959) were taken twice weekly from all plots except from those of treatment (*b*). The flotation technique was improved by the addition of an anti-foam



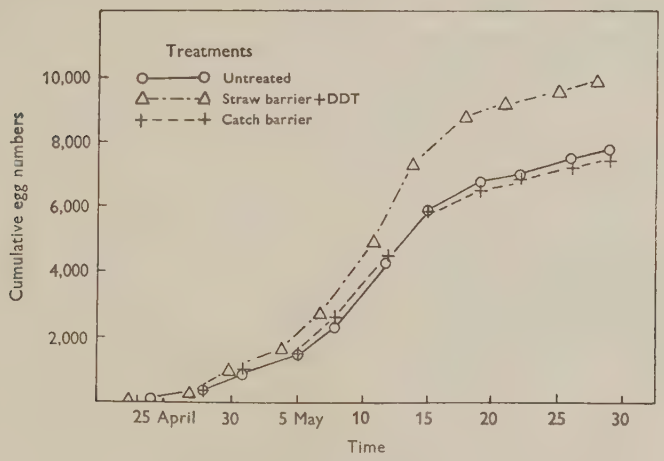
Text-fig. 2. Percentage of wilted plants occurring on different treatments. Cauliflowers, Wellesbourne, 1959.



Text-fig. 3. Numbers of *Bembidion lampros* (Bouché) trapped on different treatments. Cauliflowers, Wellesbourne, 1959.

emulsion to the water, facilitating the easier removal of the eggs. Ten plants per plot were sampled for eggs in late April, but when, in May, egg numbers became very high, only five plants per plot were used. The beetle and egg counts were carried out in the north-west quadrant of each plot so that the remaining seventy-five plants were undisturbed and could be used for plant-yield and root-infestation data.

Bembidion lampros was the beetle most frequently caught throughout this experiment: the numbers trapped on the various treatments are shown in Text-fig. 3. Within the straw-barrier DDT plots the beetle density was very much lower than on the untreated plots; while the dieldrin plant treatment caused a 51·4% reduction.



Text-fig. 4. Numbers of cabbage root-fly eggs on different treatments. Cauliflowers, Wellesbourne, 1959.

Although greater numbers of beetles were trapped on the plots surrounded by the catch barrier than on the untreated plots, the difference was not significant. On both these treatments, however, the numbers taken were significantly greater than on either the straw-barrier DDT plots or the dieldrin plant treatment. The latter treatments were also significantly different from each other in the numbers of beetles trapped.

The numbers of root-fly eggs found by the end of May on the three treatments sampled are shown in Text-fig. 4. The lowest numbers of eggs were found on plots harbouring the greatest numbers of beetles and vice versa (Text-figs. 3, 4). There was a negative correlation ($r = -0{\cdot}6983$) between the total numbers of eggs and beetles recorded by the end of May.

To determine if the different numbers of eggs occurring on the various treatments would be reflected in different numbers of larvae damaging the crop, samples of plant-root systems and adjoining soil were taken as described by Hughes (1960). The samples were taken from around nine plants on each plot, and the root-fly

larvae and puparia were extracted by flotation. Table 3 gives the estimated numbers of puparia and larvae per plant at maturity. The two sets of samples were separated by an interval of 1 week and gave very similar estimates of cabbage root-fly numbers.

The total population of larvae and puparia of cabbage root fly on the catch-barrier treatment was significantly lower than on both the untreated and the straw-barrier DDT plots; while reference to Table 2 shows that the yield of the various treatments is inversely related to the larval and pupal infestation.

Finally, after harvesting, thirty root systems from each plot were graded for the degree of damage caused by the larvae of the root fly (Wright, 1953). The damage was greatest on the straw-barrier DDT plots, and less on the catch-barrier plots than on those untreated. Such differences are again a direct reflexion of the differences in larval population.

Table 3. *Numbers of puparia and large larvae/plant (at maturity), Wellesbourne, 1959*

	Sample I: 5 June				Sample II: 12 June			
Stage treatment	Puparia	Larvae	Total	Analysis log $(n+1)$	Puparia	Larvae	Total	Analysis log $(n+1)$
(*a*) Untreated	41·8	7·6	49·4	1·10	52·9	6·7	59·6	1·27
(*b*) Dieldrin plant treatment	8·4	4·4	12·8	0·64	4·9	9·8	14·7	0·64
(*c*) Catch barrier	25·8	4·4	30·2	0·95	18·7	0·0	18·7	0·74
(*d*) Straw barrier + DDT	54·2	10·7	64·9	1·17	59·6	0·9	60·5	1·26
	S.E. = ±0·12, L.S.D. (P = 0·05 %) = ±0·22, $d > c, b$, $b < a, c$				S.E. = ±0·13, L.S.D. (P = 0·05 %) = ±0·23, $d > c, b$, $a > b, c$			

Discussion

These experiments have shown that the predators of the cabbage root fly play a very important part in the natural control of this insect. Where the numbers of beetles were decreased, greater numbers of the pest survived and the crop damage was increased. Although the cabbage root fly is killed in all its stages, the attack on the egg stage is of outstanding significance and largely determines the subsequent population level. The most important egg predator in these experiments was *Bembidion lampros*, but when its numbers began to decline in June, other species of beetles (e.g. *Trechus quadristriatus* and *Aleochara bipustulata* (Linn.)) became common and appeared to play an increasingly important role as predators later in the season. The use of insecticides in the soil for the control of this pest, although economically necessary, does appear to kill some of the predatory beetles. Such losses, however, can be reduced to a minimum by restricting the use of insecticides, wherever possible, to the soil immediately around the plant.

Fig. 1

Fig. 2

Fig. 3

WRIGHT, HUGHES AND WORRALL—*Effect of predators on cabbage root fly*

(*Facing p.* 763)

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EXPLANATION OF PLATE 12

The photographs show different levels of cabbage root-fly damage in the 1958 field experiment. Four subplots make up each plot.

Fig. 1. Foreground, straw-barrier DDT plot. Most of the plants are killed or severely stunted by cabbage root fly. Background, untreated plot. A few plants are killed or severely stunted.

Fig. 2. Untreated plot. A few plants are killed or severely stunted in each subplot. Varying degrees of stunting shown in the remainder.

Fig. 3. Dieldrin plant treatment. All plants are vigorous in growth and nearly uniform in size.

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VARIETAL RESISTANCE OF LETTUCE TO ATTACK BY THE LETTUCE ROOT APHID, *PEMPHIGUS BURSARIUS* (L.)

By J. A. DUNN

National Vegetable Research Station, Wellesbourne, Warwick

(With Plate 13)

Striking varietal differences in susceptibility to attack by the lettuce root aphid, *Pemphigus bursarius* L., were first found in lettuces grown at Wellesbourne in 1955. Subsequent work has confirmed that White Favourite and Imperials E-4 and 19551-M are highly resistant; Imperial 45634-M, Continuity and Iceberg are markedly resistant. Midas, Salad Bowl and Imperial 4164 each appear to be a mixture of susceptible and resistant plants.

Immigrant winged forms of *P. bursarius* showed no preference for colonizing any particular variety of lettuce, and it seems that resistance to attack results from antibiosis.

It is suggested that varietal differences in the composition of root sap may account for the differences in susceptibility to lettuce root aphid, but analyses of water and alcohol extracts of root sap from resistant and susceptible lettuce varieties have not shown consistent differences.

In the summer of 1955 the incidence of lettuce root aphid, *Pemphigus bursarius* L., was extremely high and striking differences in susceptibility to attack by this pest were found among 125 varieties or strains of summer lettuce growing at Wellesbourne. Some of the varieties were killed or severely damaged by *P. bursarius*, while others supported few aphids on their roots and remained almost, if not entirely, undamaged (Pl. 13, figs. 1–4). Resistant varieties were found among all the four groups of lettuce given by Watts (1955) i.e., All the Year Round group; White Boston group; Curly Crisp group and Leaf Lettuce group, and these have provided material for subsequent work on resistance to root aphid.

Experiments establishing varietal differences in resistance

In June and July migrants (fundatrigeniae) of *P. bursarius* fly from galls on poplar to lettuce where they found colonies of root-feeding aphids (Dunn, 1959). To compare the resistance of lettuce varieties to root aphid they were grown outdoors at the time of aphid migration, but because of the uncertainty of an aphid attack in the field, lettuce varieties were also grown under cages and artificially exposed to migrants of *P. bursarius*.

Field experiments 1956

Seventeen varieties of lettuce, listed in Table 1, were grown in four randomized blocks in 1956. With the exception of Imperial 5506–1, these varieties had shown either high resistance or high susceptibility to root aphid in 1955. The seed was

sown on 18 May and after thinning, each plot contained fifteen plants of one variety 15 in. apart.

Alatae of *P. bursarius* began migrating on to the lettuce at the end of June, and on several occasions in July the alatae on foliage of some varieties were counted. Counts were made on all varieties on 10 July (see Table 1).

TABLE 1. *Numbers of* Pemphigus bursarius *migrants on the foliage of lettuce varieties in July and the percentage of root systems found attacked by the aphids on 22 August* 1956

Variety		No. of alatae per ten plants, July								Total in eight counts	% root attack
		4	6	9	10	11	12	18	24		
All the Year Round group											
All the Year Round	S	0·8	0	4·3	15·9	11·3	9·7	7·3	15·6	64·9	66·7
Green Mignonette	S	—	—	—	13·9	—	—	—	—	—	72·7
Cazard elite qualitat	S	—	—	—	7·8	—	—	—	—	—	79·2
White Favourite	R	0·4	0·2	4·5	5·4	7·4	10·3	7·8	7·6	43·6	12·5
White Boston group											
Cobham Green	S	0·5	0	1·1	5·1	5·8	7·6	5·6	6·8	32·5	70·8
Midas	R	0·5	0·2	5·5	9·8	12·5	10·6	10·0	7·9	57·0	58·3
Curly Crisp group											
Webb's Wonderful	S	0·9	0·3	12·1	10·1	12·3	8·8	2·0	6·8	53·2	62·5
Imperial 20609-M	S	—	—	—	15·1	—	—	—	—	—	79·7
Imperial 21174-M	S	—	—	—	9·4	—	—	—	—	—	86·5
Imperial 5506-1	?	—	—	—	10·0	—	—	—	—	—	66·7
Imperial 847	S	—	—	—	6·4	—	—	—	—	—	91·7
Imperial 44	S	0·9	0	10·0	14·3	15·7	21·9	7·5	10·5	80·7	87·1
Imperial 19551-M	R	0·8	0·2	5·7	6·2	8·1	4·4	5·0	10·6	41·0	4·2
Imperial 45634-M	R	—	—	—	6·1	—	—	—	—	—	0
Imperial 4164	R	—	—	—	8·6	—	—	—	—	—	70·8
Imperial E-4	R	0·9	0	4·0	8·5	7·7	9·1	5·0	12·8	48·0	29·7
Leaf Lettuce group											
Salad Bowl	R	0·9	0·1	8·4	9·3	14·3	8·1	5·7	15·6	62·4	41·7

S, susceptible in 1955. R, resistant in 1955. ?, new untested strain with the wild *Lactuca virosa* as one parent; supplied by Dr R. C. Thompson of the U.S. Department of Agriculture.

Table 1 indicates that similar numbers of alatae occurred on the foliage of all varieties, showing that there was no preferential selection.

On 22 August, 3 weeks after the last alata was seen on the lettuce, the roots of six plants from each plot were examined for colonies of *P. bursarius*. Table 1 shows that the ultimate root aphid attack on the varieties listed ranged from 0 to 92 %. Such differences cannot be explained in terms of varietal preferences by the insect and would appear to arise because the varieties supporting the fewest aphids are biologically unsuitable as hosts (antibiosis; Painter, 1951).

Table 2 gives the total number of aphids on the root systems of twenty-four plants of each variety on 22 August, together with the aphid numbers on the roots of the varieties grown in two smaller experiments nearby. These smaller experi-

ments (Exps. 2 and 3) both comprised ten pot-grown plants per variety, one plant of each variety being positioned at random in each of ten rows. They were sampled for root-attacking *P. bursarius* on 23 August.

TABLE 2. *Populations of* Pemphigus bursarius *on the roots of lettuce varieties in field experiments,* 1956

Variety		Plot experiment: aphids per 24 plants	Random plantings: aphids on 10 plants (Exp. 2)	Random plantings: aphids on 10 plants (Exp. 3)
All the Year Round	S	1320	132	251
Green Mignonette	S	3064	—	—
Cazard elite qualitat	S	3293	832	—
White Favourite	R	118	58	0
Cobham Green	S	1393	238	585
Midas	R	1017	542	0
Webb's Wonderful	S	795	1761	656
Imperial 20609-M	S	1183	152	607
Imperial 21174-M	S	3570	841	—
Imperial 5506-1	?	791	931	—
Imperial 847	S	3884	1104	—
Imperial 44	S	3586	935	2167
Imperial 19551-M	R	58	2	0
Imperial 45634-M	R	0	2	2
Imperial 4164	R	2266	134	529
Imperial E-4	R	44	2	11
Salad Bowl	R	995	20	1066

S, susceptible in 1955. R, resistant in 1955. ?, new untested strain with the wild *Lactuca virosa* as one parent; supplied by Dr R. C. Thompson of the U.S.D.A.

In 1956 the populations of *P. bursarius* were very low compared with those of 1955, but the high degree of resistance to root aphid shown in 1955 by White Favourite, Imperials 19551-M, 45634-M and E-4 is again evident. Imperial E-4 is highly resistant to root aphid (now acknowledged as being *P. bursarius*) in California, and Lange (1955) stated that in a trial at Salinas, California, the number of aphids per root system averaged nine on Imperial E-4 and 688 on the variety Great Lakes. In 1955 at Wellesbourne, Great Lakes was similar in susceptibility to Imperials 847 and 44: a comparison of the aphid numbers shown for these varieties in Table 2 with the numbers on E-4 indicates that the resistance of E-4 at Wellesbourne paralleled that in California.

1957 *experiment*

In 1957 a plot of each variety listed in Table 3 was grown in each of five randomized blocks. Each plot contained nine plants of one variety 15 in. apart. The plants were sown in May, and on 29 June *P. bursarius* alatae began migrating into the plots. Table 3 gives the populations of root aphids found on 7 August on three diagonally placed plants in each plot.

The aphid populations were again low but the results confirm the high resistance

of White Favourite, Imperials 19551-M and E-4. The varieties Continuity (found to be resistant to root aphid in 1955) and Iceberg, are both stated in seed catalogues to be resistant to drought. These varieties supported only very small aphid populations, and this resistance to *P. bursarius* might explain why they appear to withstand drought, as in the hot dry summer of 1955.

TABLE 3. *Populations of* Pemphigus bursarius *on the root of lettuce varieties in field experiment,* 1957

Variety		Aphids on 15 plants
All the Year Round group		
All the Year Round	S	1884
Bonte Chili	S	228
White Favourite	R	7
Continuity	R	48
White Boston group		
Borough Wonder	S	490
Midas	R	92
Curly Crisp group		
Webb's Wonderful	S	1647
Imperial 847	S	1115
Imperial 19551-M	R	4
Imperial E-4	R	0
Iceberg	R?	4
Leaf Lettuce group		
Salad Bowl	R	285

S, susceptible in 1955. R, resistant in 1955. R?, listed in seed catalogues as drought resistant.

On 2 July, a day very suitable for the immigration of *P. bursarius*, counts were made of the alatae on all plots of Continuity, on the plots adjoining Continuity, and on Borough Wonder. It was thought possible that the alatae of *P. bursarius* might prefer the yellow-green of Borough Wonder to the reddish-bronze foliage of Continuity, but the figures below show no real difference in the numbers of alatae, so it seems that the resistance shown by Continuity to root aphid attack is not the result of preference.

	No. of alatae
Continuity	22
Borough Wonder	27
Lettuce in plot on north side of each Continuity plot	27
Lettuce in plot on south side of each Continuity plot	16

Experiments under cages

Cages were made in which the varieties to be tested for resistance could be enclosed with alatae of *P. bursarius* in such a way that any selection by the aphids was not dependent on the degree of illumination of the plants. It was essential

to use alatae from poplar galls, so as to avoid lettuce-bred aphids that might favour a particular lettuce variety on the basis of familiarity. This limited the work to a time when alatae from poplar galls were readily available in the field.

The dome-shaped cages, 30 in. diam. and 18 in. high, were of polythene with Tygan mesh at the top, and the supporting galvanized wire presented minimum obstruction to light. They were spaced out on unsheltered bare ground.

Each cage contained five plants of each of five or six varieties placed in random order within a basic layout. The plants were in 3-in. pots which were sunk in the ground so that the rims were just covered and a flat surface of soil was obtained.

TABLE 4. *The relative susceptibilities of lettuce varieties to root attack by* Pemphigus bursarius, *based on the size of the aphid populations supported by each variety; the most susceptible variety in each cage being rated at* 100

Variety		Cages 1	2	3	4	5	6	7	8	9	10	Mean
All the Year Round group												
All the Year Round	S	87	—	80	100	—	—	94	95	—	—	91
Bonte Chili	S	—	100	—	0	—	100	—	—	—	—	66
White Favourite	R	2	11	—	8	—	—	14	0	—	—	7
Continuity	R	—	19	—	—	0	14	—	—	—	—	11
White Boston group												
Borough Wonder	S	—	—	48	—	—	—	—	—	100	100	83
Midas	R	—	—	100	—	33	—	3	0	—	—	33
Curly Crisp group												
Webb's Wonderful	S	100	69	—	72	—	—	95	100	—	—	87
Great Lakes	S	—	—	—	—	—	—	100	93	—	—	96
Imperial 847	S	—	—	59	—	100	—	—	—	100	69	82
Imperial 44	S	67	—	—	—	—	—	—	—	—	—	67
Imperial 19551-M	R	1	—	—	—	—	—	—	—	9	8	6
Imperial 45634-M	R	—	—	—	—	—	—	—	—	13	14	13
Imperial E-4	R	3	2	—	8	—	0	—	—	3	9	6
Iceberg	R?	—	—	1	—	9	—	—	—	—	—	5
Leaf Lettuce group												
Salad Bowl	R	—	—	—	—	2	7	—	—	—	—	4

S, susceptible in 1955. R, resistant in 1955. R?, listed in seed catalogues as drought resistant.

Three batches, each of approximately 200 alatae (freshly issued from poplar galls), were introduced into each cage within a few days of its establishment. They were placed in a small open container suspended centrally 5 in. from the top. The lettuce root systems were examined 5 weeks later and the aphid numbers recorded. Table 4 shows, for each cage, the aphid population on each variety as a percentage of the population on the most heavily attacked variety within the cage. A measure of varietal susceptibility is thus shown; the most susceptible variety being rated at 100.

The results confirm previous findings: all the varieties shown in field experiments

to be resistant supported under 20%, and generally less than 10%, of the aphid numbers found on susceptible varieties.

The agreement between cage and field results adds further evidence that antibiosis and not preference determines the consistent varietal differences in the size of the root aphid populations, because any preference for certain plants by the alatae, would, for reasons of plant spacing (Dunn, 1959) express itself more clearly in the field than under the cages.

Investigation into the factors conferring resistance

Painter (1951) reviewed the factors associated with resistance and doubted whether structural differences between plants, such as thickness or hardness of epidermis could be sufficient to permit an aphid to feed on one plant but prevent it from feeding on another. Similarly, in lettuce varieties, no morphological difference has been found that could explain why some lettuce are most resistant than others.

It is most likely that resistance to *Pemphigus bursarius* arises from differences in the sap of resistant varieties compared with that of susceptible varieties.

Chemical analyses have been done on both water and alcohol extracts of the sap from roots of each of the lettuce varieties listed in Table 1. The sap in all cases came from whole root systems of plants of similar age and size (12th leaf stage) which had been grown under standard conditions in a glasshouse.

No consistent differences were found either in the colour or the pH of the sap extracts from resistant and susceptible varieties. Similarly, using paper chromatographic methods, no consistent qualitative differences between resistant and susceptible varieties were found in the sap when analysed for amino acids, phenols or sugars.

The sap of each of three resistant varieties (White Favourite, Imperials 19551-M and E-4) and each of three susceptible varieties (Green Mignonette, Borough Wonder and Imperial 847) has been analysed for quantitative differences in sugars, amino acids and total dry matter. All plants used had been uniformly grown and the root sap was obtained and analysed at two different stages of plant growth (i.e. at the 9th and at the 16th leaf stage). However, no consistent results were obtained on which a possible explanation for resistance could be based.

Discussion

The varieties White Favourite and Imperials E-4 and 19551-M consistently showed similar and high resistance to lettuce root aphid attack. Imperial 45634-M, Continuity and Iceberg were also markedly resistant. In experiments in 1956 Midas showed a mixture of resistant and susceptible plants. Midas is a variety not more than ten generations old (R. C. Thompson, in litt.), bred under conditions where root aphid is not a serious pest.

Two other new American varieties, Imperial 4164 and Salad Bowl, similarly have shown a mixture of resistant and susceptible plants, though on all occasions Salad Bowl supported fewer aphids than did the most susceptible varieties. In 1955

it showed no symptoms of damage by root aphid (Pl. 13, fig. 3) but more aphids were present on the root systems of Salad Bowl than on most other resistant varieties.

Bonte Chili was exceptional among the susceptible lettuces in that the cage experiments showed that plants very resistant to root aphid occurred in this variety.

The negative results from the sap analyses do not necessarily rule out the existence of differences between root saps of resistant and susceptible varieties. Kennedy, Ibbotson & Booth (1950) showed that the leaves of *Euonymus europaeus* and *Beta vulgaris* were colonized by *Myzus persicae* and *Aphis fabae* according to their suitability in terms of physiological age. Thus because the susceptibility of plants to aphid attack can vary with the stage of plant maturity, sap analyses done at one particular stage of plant growth may or may not coincide with the time of maximum susceptibility or resistance of the plants to attack. There will certainly be both varietal and individual plant differences in the time that peak resistance is reached among lettuces known to be resistant to root aphid attack. Similarly, there will be differences in peak susceptibility. The analyses of root sap from individual lettuce plants which at the time of sampling are showing high susceptibility or resistance to root aphid, might help to overcome this problem.

The help given by Mr L. E. Watts, on whose variety trial in 1955 the initial observations on resistance were made, is gratefully acknowledged. Thanks are also due to Drs S. P. Spragg and J. Clare Hughes for analysing the extracts of root sap, to Dr T. W. Whitaker of U.S. Horticultural Field Sta., La Jolla, California, for supplying seed of Imperials 19551-M, 45634-M, 20609-M and 21174-M and to Dr R. C. Thompson of U.S. Agricultural Research Sta., Salinas, California, for supplying seed of Midas, Salad Bowl and Imperials 4164 and 5506–1.

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EXPLANATION OF PLATE 13

Photographs taken during a severe root-aphid attack at Wellesbourne in 1955, showing the performance of some varieties of summer lettuce which are highly resistant to the aphid compared with susceptible lettuce varieties which succumbed to the attack.

Fig. 1. Left, White Favourite (resistant); right, Romaine blonde maraîchère (susceptible).

Fig. 2. Left, Rolico (susceptible); right, Imperial E-4 (resistant).

Fig. 3. Left, Salad Bowl (resistant); right, Webb's Wonderful (susceptible).

Fig. 4. Foreground, Imperial 19551-M (resistant); middle and background, Imperials 847 and 44 (susceptible).

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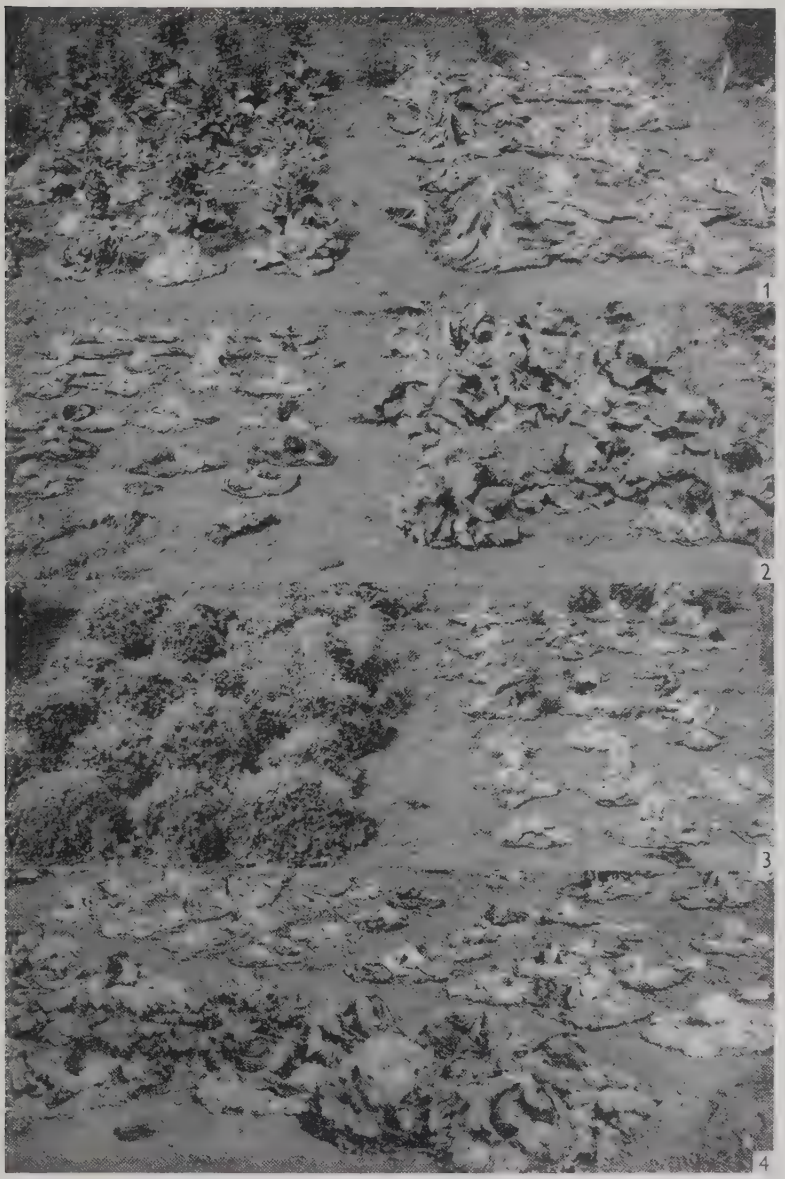

DUNN—*Resistance of lettuce to* Pemphigus bursarius

(*Facing p.* 770)

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STUDIES ON THE IMPORTANCE OF WILD BEET AS A SOURCE OF PATHOGENS FOR THE SUGAR-BEET CROP

By A. J. GIBBS

Rothamsted Experimental Station, Harpenden, Herts

(With 3 Text-figures)

Beet yellows virus, beet mosaic virus, rust (*Uromyces betae* (Pers) Lév.), and downy mildew (*Peronospora schachtii* Fuckel.) were found to be common in wild beet (*Beta vulgaris* s.-sp. *maritima* L.) growing on the foreshores of south Wales and southern England. The virus diseases were more prevalent in south-east England than in the west, rust more in the west than in the east, and downy mildew is equally prevalent in all regions.

Beet yellows is the most commercially important disease and is more common in sugar-beet crops in East Anglia than elsewhere in Great Britain. There was no evidence that beet yellows spread in East Anglia from wild beet to nearby sugar-beet crops during the springs of 1958 or 1959, and *Myzus persicae* Sulz., the principal vector of yellows, was rarely found on wild beet growing on the foreshore.

In glasshouse experiments aphids colonized sugar-beet plants watered with tap water in preference to those watered with sea water. Daily watering with sea water made plants unpalatable to aphids within 14 days. Aphids also preferred leaves sprayed with distilled water to those that had been sprayed with sea water. Salt solutions gave results similar to those obtained with sea water.

Wild beet (*Beta vulgaris* s.-sp. *maritima* L.) is a common littoral plant of north-west Europe. It is the European form of the highly polymorphic common beet (*B. vulgaris* L.), which, with various polyploids, grows throughout the temperate zone of the northern hemisphere (Hector, 1938; Bell, 1948). *B. vulgaris* is thought to have its centres of diversity in the eastern and central Mediterranean, and was there first used as a green vegetable. Cultivated strains were subsequently developed as sugar beet, mangold, fodder beet, beetroot, spinach beet, and chard (Bell, 1948; Darlington, 1956).

Wild beet, first recorded in England in 1692, has since been found in all the seaboard counties of the British Isles south of latitude 56·5° N. (Druce, 1932). It is common on the foreshores of the southern counties, growing mainly in the few feet above spring high-tide level, especially in two types of habitat: (1) on shingle banks, where its ability to withstand salt water and its extensive root system enable it to compete successfully with other species; (2) on soil newly exposed by coastal erosion, or on new sea walls. Wild beet produces many corky fruits (Turrill, 1948), which are water dispersed, germinate quickly and enable the species to be among the first to colonize newly exposed soil. Grasses, which later invade such stands of wild beet, often prevent the wild beet from regenerating after the first beet plants die; in undisturbed communities the wild beet are largely succeeded

by grass species in 3–5 years, and become a minor component of the plant community.

The cultivated varieties of *B. vulgaris* are susceptible to all the diseases of the wild forms. Four of the commonest diseases of sugar beet are: beet yellows (Quanjer & Roland, 1936); beet mosaic (Quanjer & Roland, 1936); rust (*Uromyces betae* (Pers.) Lév.) (Hull, 1949) and downy mildew (*Peronospora schachtii* Fuckel) (Salmon & Ware, 1926). All these diseases also occur in wild beet, but beet yellows is easily the most important disease, because *Myzus persicae* Sulz. often spreads the virus rapidly and extensively (Watson, Hull, Blencowe & Hamlyn, 1951) and infection can greatly decrease the yield of sugar (Watson, Watson & Hull, 1946). Although less important, the other diseases sometimes cause considerable local losses in some years.

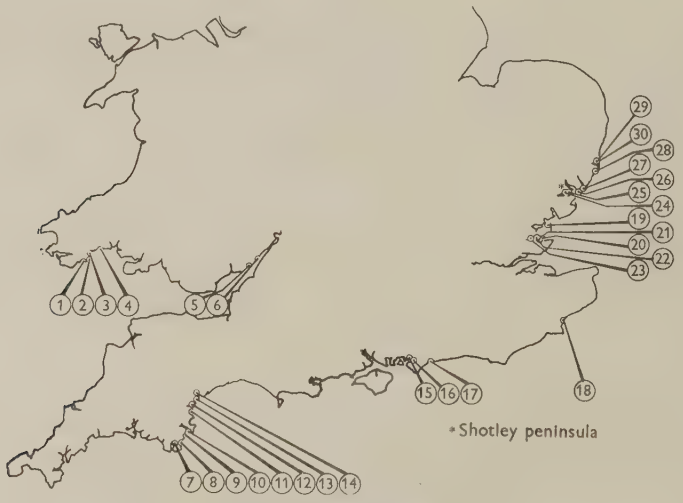

Fig. 1. Location of sites in wild-beet survey.

RESULTS

Survey of pathogens in wild beet

During the autumn of 1958 and summer of 1959 the incidence of the four pathogens listed above was recorded in stands of wild beet at the thirty sites on the foreshores of Wales and southern England shown in Fig. 1. At each site ten to fifty plants were examined visually for symptoms.

Estimates of disease incidence based on symptoms may be underestimates, as symptoms of some diseases are influenced by host genotype (Watson & Russell, 1956), and by such factors as shading, nutrition, etc. (Hull & Watson, 1947; Watson, 1955). Gibbs & Gower (1960) found that visual estimates of yellows incidence in two stands of wild beet (44 and 12%) were lower than the estimates deduced from transmission experiments (64 and 15%). By contrast, the incidence

of beet mosaic virus infection can be accurately estimated by inspection. Thus when saps from leaves of eighty wild-beet plants were tested by inoculation to leaves of *Chenopodium amaranticolor* Coste & Reyn, the transmissions agreed with the visual estimates of infection. Estimates of fungus disease incidence are likely to be accurate, but this fact could not be checked. Fig. 2 shows the estimates of disease incidence with the individual sites grouped into six regions, with region A the westernmost and region F the easternmost. On average nearly half the wild-

Fig. 2. Survey of four diseases of wild beet. Region A, south-west Wales coast, sites 1 to 4. Region B, Devon coast, sites 7 to 14. Region C, Severn Estuary, sites 5 and 6. Region D, Sussex and Kent coast, sites 15 to 18. Region E, Essex coast, sites 19 to 23. Region F, Suffolk coast, sites 24 to 30. M, the mean regional disease incidence. Em, the mean disease incidence for estuarine sites. Cm, the mean disease incidence for coastal sites. S.E. %, the standard error of disease incidences between sites within regions expressed as a percentage of the regional mean.

beet plants had beet mosaic, one-third yellows, one-quarter rust, and one-tenth downy mildew. Also about one-third were infected with leaf-spotting fungi such as *Ramularia beticola* Fautr. & Lamb., and *Cercospora beticola* Sacc., and one fifth were infested with the larvae of the mangold fly, *Pegomyia betae* Curt.

Analysis of the records of individual wild-beet plants showed that only 51% of

the expected number of plants were infected with both beet yellows virus and rust. This value differs significantly from the expected ($P = 0{\cdot}01$). Perhaps doubly infected beet die, or infection with one pathogen may interfere with infection by or mask the symptoms caused by the other, though no evidence was found to support any of these hypotheses. No other pairs of pathogens showed comparable interactions, indeed the two virus diseases seemed to infect plants quite independently although they are presumably spread by the same aphid species.

Both virus diseases were more prevalent in the eastern than in the western regions (Fig. 2). The variation of the disease incidence between sites is indicated by the standard error percentage (S.E.%) of the site estimates. Thus the low S.E.% of the disease estimates of the two virus diseases indicates that these diseases are distributed relatively uniformly within regions, and are presumably not influenced by local conditions. That both virus diseases have similar S.E.%'s, though mosaic is more prevalent, suggests that both viruses depend on the same factors for spread between sites (e.g. the same vector), but mosaic is more easily spread within each site. However, the incidence of beet yellows virus was probably underestimated. About half the sites were estuarine with land on two or more sides, and the mean incidences of virus diseases there were similar to those of coastal sites (Fig. 2).

Rust was commonest in the west. The higher S.E.% suggests that its spread is more influenced by local conditions than is the spread of the viruses. The mean coastal site rust incidence was higher (28%) than the mean estuarine site incidence (18%), though rust was most prevalent in the southern and western sites where most sites were coastal.

Downy mildew was equally prevalent in all regions but local variation was great. The mean incidence in estuarine sites was 11% and coastal sites 6%. This difference was not statistically significant ($P = 0{\cdot}05$).

Spread of virus diseases from wild beet to nearby sugar-beet crops

Sugar-beet crops in the south-east part of East Anglia usually have more plants infected with beet yellows virus than do crops in other parts of Great Britain, which suggests that in that area there is either a potent primary source of the disease (e.g. wild beet) or there is more secondary spread of the virus perhaps due to climatic conditions favouring the multiplication and movement of the vector.

Blencowe (1956) found that the incidence of beet mosaic and downy mildew in sugar-beet crops on the Shotley peninsula of Suffolk (Fig. 1) was positively correlated with proximity to wild beet growing on the foreshore, suggesting that wild beet was a source of these two diseases. Though he found aphids on wild beet in the spring of 1957, he found none in 1955, and he found no direct evidence that beet yellows virus spread from the wild beet to sugar-beet crops (Blencowe, 1955, 1957).

During the spring and early summer of 1958 and 1959 several sites on the Shotley peninsula of Suffolk, where commercial sugar-beet crops were growing

near foreshore infested with wild beet, were regularly observed, to assess the extent to which beet yellows spread from the wild beet to the sugar-beet crops (Gibbs, 1958). A few *Myzus persicae* were found on wild beet at one site in late 1957, but in 1958 despite frequent and extensive inspections none was found, though *M. persicae* was found on nearby sugar-beet crops in the first week in June and a fortnight later the aphid population in these crops was about 0·5 aphids per plant. Alate aphids were caught on sticky yellow cylindrical aphid traps (Broadbent, Doncaster, Hull & Watson, 1948) above the sugar-beet crops from the beginning of May, whereas aphids were not trapped above wild beet until a fortnight later.

Healthy sugar-beet plants in pots were placed among wild beet at two sites and left there for 2 weeks when they were replaced by others. After their 2 weeks in the open, plants were sprayed with insecticide and then kept in an insect-free glasshouse to see whether they developed symptoms. Of 600 such plants exposed during a 10-week period starting 8 weeks before beet yellows showed on nearby sugar-beet crops, only one developed yellows and three developed mosaic virus. Symptoms of yellows appeared on sugar-beet plants in crops near the wild beet 2 weeks before any sugar beet showed mosaic, although 60–80% of the nearby wild beet had mosaic virus symptoms and 30–50% had yellows symptoms.

In 1959 *M. persicae* was found multiplying on wild beet at only one of eight sites on the Shotley peninsula. At this site aphids were colonizing only plants at least 20 ft. above the spring high-tide level. This observation and those made in 1958 suggested that *M. Persicae* do not infest those wild beet growing at or near high-tide level, whereas wild beet growing some distance from high-tide level, and the nearby sugar-beet crops, are readily infested.

The effect of treating sugar-beet plants with sea water on subsequent colonization by Myzus persicae

Experiments were done to see whether the colonization of sugar-beet plants by apterous *M. persicae* is affected by sea water, when watered on to the soil around the roots or sprayed on to the leaves.

Four different treatments were applied to groups each of one dozen sugar-beet seedlings (variety Klein E) growing in pots of John Innes compost. Each group was watered daily for 1 month with either undiluted sea water, sea water diluted 1/2, sea water diluted 1/4, or with tap water. Ten apterous *M. persicae* were then put on to each plant of half of each group and all pots were arranged in random positions in a close hexagonal packing pattern, so that each pot touched the six nearest pots. One week later the aphids on each plant were counted.

The number of aphids found was 227% of the number of aphids put on the plants. The aphids had moved freely from plant to plant for there were only 8% more aphids on those plants initially infested than on the others. However, only 4·6% of the total number of aphids were on those plants watered with undiluted sea water; plants watered with diluted sea water were infested to the same extent

as those watered with tap water. Plants that had been watered with sea water for 1 month had only half the leaf area, and were darker green, than plants watered with tap water.

To find how long sugar-beet plants must be treated with sea water to make them unpalatable for aphids, three groups of sugar-beet plants were each watered daily with undiluted sea water, sea water diluted 1/2, and tap water. Every 7 days half of the plants in each group were each infested with ten aphids and all were put as already described so that aphids could crawl freely from plant to plant. After

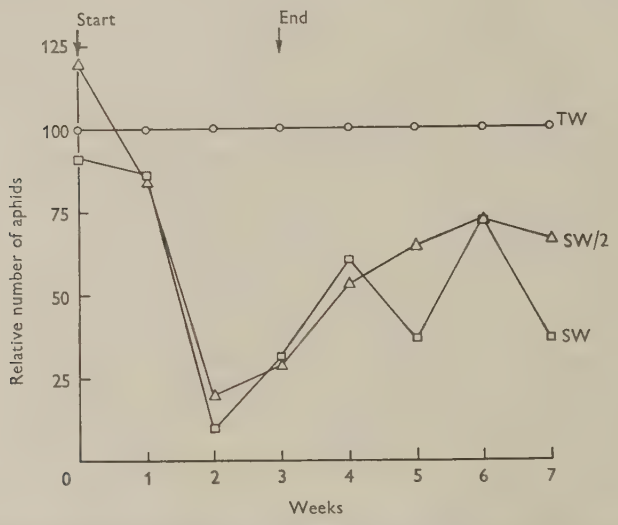

Fig. 3. Effect of watering sugar-beet plants with sea water on subsequent colonization by apterous *M. persicae*. TW, plants watered with tap water. SW, plants watered with sea water. SW/2, plants watered with a mixture of equal volumes of sea water and tap water. TW, SW, and SW/2 treatments were given for 3 weeks between START and END, then all plants were watered with tap water. Relative number of aphids is given by: $100\ (\Sigma \log (n_1+1)/\Sigma \log (n_0+1))$, where n_1 is the number of aphids on each of the SW or SW/2 plants, and n_0 is the number of aphids on each of the tap-water plants.

24 hr. the aphids on each plant were counted and all the plants were sprayed with a 0·1% solution of the non-persistent systemic insecticide phosdrin (2-carbomethoxy-methylvinyl dimethyl phosphate). After watering with sea water for 3 weeks, all plants were watered with tap water and the aphid preference tests continued for a further 4 weeks.

Only 32% of the aphids put on to the plants were found 24 hr. later, of which those plants initially infested had only 11% more than the others. Both undiluted and diluted sea water decreased colonization by aphids, the effect appearing within a fortnight of starting the differential watering, and persisting at least 4 weeks after

it ended (Fig. 3). The effect of sea water may be partially due to the decrease in leaf area which it causes. Salt solutions containing salts in the same concentrations as sea water (Challenger Staff, 1882–95) affected aphid colonization in the same way as did sea water.

To see whether spraying with sea water affected aphid colonization, sugar-beet plants were trimmed to leave the four youngest fully expanded leaves on each plant. Both faces of half of each leaf were sprayed with sea water or salt solutions, and the other half of each leaf was sprayed with distilled water. When the leaves were dry, five apterous *M. persicae* were put on each leaf. When the aphids on each surface of both halves of every leaf were counted 24 hr. later, none were on the adaxial surfaces of the leaves. Only 30% of the aphids found were on half-leaves sprayed with sea water or salt solutions. The solids from evaporated sea water were hygroscopic, and sprayed leaves could not be kept dry all the time, so aphid colonization may have been affected because the aphids were repelled by the water droplets.

Discussion

As a high proportion of wild beet is diseased, it obviously provides a potential source of infection for sugar-beet crops. I did not study the spread of fungus diseases from the wild beet, but other workers have shown that such spread occurs and can cause considerable damage (Blencowe, 1955, 1956, 1957; Hughes, 1959; Hull, 1949; Leach, 1945; MacKay, 1957).

Attempts to assess the importance of wild beet as a source of the two viruses is complicated by the fact that they are spread by aphids, especially *M. persicae*. A high proportion of wild beet is infected with beet yellows or beet mosaic virus, or both, so aphids presumably visit them. However, there is field evidence that aphids do not overwinter on or readily colonize wild beet growing near the high-tide level. This may be because wild beet growing on the foreshore is often sprayed or watered with sea water (Boyce, 1954), and experimental evidence shows that aphids dislike beet treated in these ways.

Since aphids rarely overwinter on wild beet, it is probably not an important primary source of viruliferous aphids in the spring. However, migrant aphids may feed on wild-beet plants long enough to transmit viruses to and from them and also to the sugar-beet crops as is suggested by the gradients in the incidence of mosaic often seen in crops growing near wild beet (Blencowe, 1956). A combination of warm calm weather, leaching rain, and neap tides could increase the suitability of wild beet for aphid colonization.

Plants infected with beet yellows virus in the glasshouse develop symptoms in 10–20 days, whereas symptoms of mosaic appear in 7–10 days. In crops growing near wild beet in 1958 and 1959, plants with yellows were found 14 days before any plants with mosaic. Aphid counts during this period showed that the first plants were infected with beet yellows virus when few or no aphids could be found colonizing the crops, and mosaic occurred only after the crops were infested and many flying aphids had been trapped above the crop. The incidence of beet mosaic virus was positively correlated with proximity to the wild beet. These results

suggest that beet yellows virus was taken into the crops by the first aphids settling on the crop, whereas beet mosaic virus was taken into the crops by the second and subsequent generations of migrant aphids.

Only in such circumstances as sea-wall building, or flooding (as in East Anglia in 1953), do wild-beet plants grow in places far enough from sea water or spray to be permanently suitable for aphid colonization, and in these situations the wild beet are usually soon succeeded by other species probably before they become a serious danger to nearby sugar-beet crops.

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THE RELATION BETWEEN INFESTATION BY THE STALK BORER, *BUSSEOLA FUSCA*, AND YIELD OF MAIZE IN EAST AFRICA

BY P. T. WALKER*

Colonial Pesticides Research Unit, Porton Down, Salisbury, England

(With 3 Text-figures)

The relation between yield and infestation by the maize stalk borer, *Busseola fusca* Fuller, is expressed as a series of regression lines for crops of different potential yields. The reasons are given for assuming that the regression is rectilinear, and that the regression coefficient b, or rate of fall in yield, is proportional to the maximum expected yield of the crop, that is that $b \propto \bar{y}$.

For the crops examined, the formula for a general regression line of yield, as hundreds of pounds of dry grain per acre, on infestation, as the percentage of plants attacked transformed to angles, is $y = 45{\cdot}1 - 0{\cdot}55x$ for a high-yielding area, and $y = 14{\cdot}55 - 0{\cdot}23x$ for a lower yielding crop. This is equivalent to a 35 lb./acre increase in grain yield for every 1 % decrease in infestation for the higher yielding group, and 17 lb. increase per 1 % decrease for the lower.

The applications of the relationship are discussed, in particular to studies of the economics of control by insecticides, and of the accuracy of methods of sampling infestations.

A series of experiments in Tanganyika on the control of stalk borer in maize (Walker, 1960) have provided data on a number of associations between infestations and yields of maize, the degree of infestation being controlled by insecticides. The range of yields obtained varied from near the local maxima to the low yields resulting from heavily attacked crops.

Trials in the poor agronomic areas of the Western Province were taken as examples of the relationship for a low-yielding crop. There, maize trials yielding $3\frac{1}{2}$ bags (E. African bag = 200 lb.) of grain per acre, yielded 7 bags (1400 lb.) per acre when stalk borer was controlled. In other trials at Mbulu, Northern Province, where cultivation and growing conditions were excellent and good varieties were planted, the control of stalk borer increased yield from 4 to 25 bags per acre.

The insect responsible was almost invariably *Busseola fusca* Fuller, although a few specimens of *Sesamia calamistis* Hmps. and *S. poephaga* Tams and Bowden may have contributed to the infestation at both places.

There are many studies on the effect of insect attack on yield, and Strickland (1956) has reviewed the problem for pests of British Agriculture, with special reference to pest density (Strickland, 1954). Similar studies on *Pyrausta nubilalis* are mentioned below, and work on the borers, *Chilo zonellus* Swin. (Pradhan & Prasad, 1955), *Bissetia steniellus* Hmps. (Kapoor, 1957) in India, and *Busseola fusca*

* Formerly with the Colonical Pesticides Research Unit, Arusha, Tanganyika, East Africa.

and other borers in West Africa by Bowden (1956), Sutherland & Gregory (1953–4) and Harris (1956–7) have also related yields to infestations. The whole problem is discussed by Jepson (1954).

The infestation rate

To maintain unity with counts of maize planted singly in rows, the percentage of individual plants attacked is taken as the most practical measure of the infestation instead of the percentage of 'hills' (groups of plants planted together) containing one or more infested plants. The latter method will inflate the infestation figure, depending on the number of plants per hill likely to be infested. The figures for Mwanhala, trial E, are therefore arbitrarily reduced by a quarter in order to compensate for this factor. This reduction is considered a sufficiently close approximation, which in any case causes little shift to the left on a line with a slope of $-0{\cdot}2$.

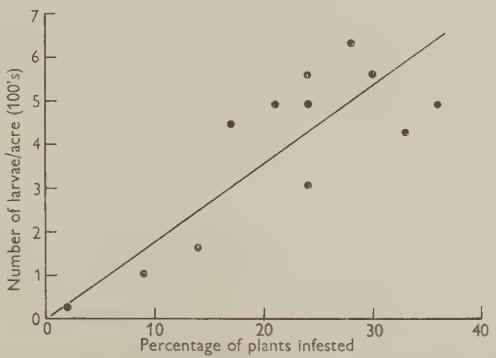

Fig. 1. The relation between the percentage of maize stalks infested and the number of stalk borers per acre. (Based on figures given by Coaker, 1956.)

The number of larvae

Few figures are available for the absolute number of borer larvae to be found per node, per plant or per acre for African borers. If a plant is infested, however, a single whole egg batch is usually laid on it and there will be sufficient larvae in the plant to have an effect on yield. No relationship would be expected such as that found by Patch, Deay & Snelling (1951) for the European corn borer, *Pyrausta*, where the number of egg batches laid on a plant can vary, and a relationship between yield and number of borers per plant is possible. With the African borer it is probable that no such relationship will be found. This applies to the primary infestation. Late infestations have not been found unduly crippling during the seasons studied. In a secondary infestation a few large migrating larvae sometimes have an effect on the yield of individual plants and hence in that case the above generalizations may not apply.

For these reasons the percentage infestation is considered an accurate guide to

the insect population, and in papers by Duerden (1953) and Coaker (1956) the number of larvae and pupae per acre is proportional to the percentage infestation for maize. The figures given by Coaker are plotted in this form in Fig. 1.

There is a need for further information on the numbers of insects per plant, the distribution of insects in the plant, and how their number changes during migration, and during the life of the plant. A similar study has been carried out by Batchelder (1949) on *Pyrausta*. As Pruthi & Pradhan (1946) have pointed out, insect populations should be given in terms of the number of plants and tillers per acre.

RESULTS

Correlation

There are five trials on stalk borer from which associated infestation and yield figures can be taken. Trials A–D took place at Mbulu, Northern Province, and trial E at Mwanhala Experimental Station, Western Province. The original data are given in Table 1, and the statistics of correlation and regression in Table 2. All percentage infestations have been transformed to angles before analysis.

TABLE 1. *Associated yields* (y) *in* 100 *lb. grain per acre and percentage of plants infested* (x)

Trial													
A	y	39	40	38	37	46	41	41	38	53	48		
	x	9	7	7	3	5	6	4	7	6	5		
	y	32	49	49	39	37	39	17	28	24	29		
	x	4	0·2	0	0·6	0·8	17	41	44	42	41		
B	y	26	35	42	35	12	22	29	28				
	x	10	9	6	3	40	30	33	27				
	y	16	23	23	25	15	8	13	16				
	x	32	27	32	31	39	72	43	41				
C_1	y	33	32	47	42	39	37	43	43	43	33	28	37
	x	31	40	7	14	14	4	4	5	7	40	39	23
C_2	y	25	26	28	29	32	32	30	37	30	19	25	23
	x	35	28	31	17	10	11	11	5	6	50	41	37
D_4	y	50	53	45	27								
	x	27	6	14	36								
D_6	y	42	49	38	39								
	x	13	4	22	33								
E*	y	7	8	11	14	8							
	x	33	23	4	1	20							

* Adjusted yields.

Trial D was superimposed on a manurial and cultivation trial which suffered high error variation, and correlation was not significant. The means of the four treatments of trial D, derived from twenty-four pairs of observations, gave the results shown in the above table. The statistics for individual blocks of this trial are of interest, however, because the significantly higher yielding blocks, 4 and 6, which

have the highest degree of correlation between yield and infestation in trial D, have regression coefficients, $-0{\cdot}87$ and $-0{\cdot}45$, respectively, in the same order as their yields, supporting the proposition that the regression coefficient, or slope of the regression line, is less with lower maximum expected yields.

TABLE 2. *Correlation* (r) *and regression* (b) *coefficients for yield as* 100 *lb. grain per acre, and percentage infestation transformed to angles*

Trial	r	P	$b \pm$ S.E.	D.F.	5% limits
A	$-0{\cdot}754$	$> 0{\cdot}001$	$-0{\cdot}50 \pm 0{\cdot}104$	18	$\pm 0{\cdot}218$
B	$-0{\cdot}879$	$> 0{\cdot}001$	$-0{\cdot}69 \pm 0{\cdot}101$	14	$\pm 0{\cdot}216$
C_1	$-0{\cdot}847$	$> 0{\cdot}001$	$-0{\cdot}435 \pm 0{\cdot}084$	10	$\pm 0{\cdot}19$
C_2	$-0{\cdot}934$	$> 0{\cdot}001$	$-0{\cdot}415 \pm 0{\cdot}050$	10	$\pm 0{\cdot}11$
D (mean)	$-0{\cdot}886$	$< 0{\cdot}1$	$-0{\cdot}48 \pm 0{\cdot}179$	2	$\pm 0{\cdot}769$
D_4*	$-0{\cdot}752$	$< 0{\cdot}1$	$-0{\cdot}87 \pm 0{\cdot}541$	2	$\pm 2{\cdot}326$
D_6	$-0{\cdot}911$	$< 0{\cdot}05$	$-0{\cdot}45 \pm 0{\cdot}143$	2	$\pm 0{\cdot}617$
E	$-0{\cdot}972$	$> 0{\cdot}01$	$-0{\cdot}23 \pm 0{\cdot}03$	3	$\pm 0{\cdot}101$

* D (other blocks): poor yield and correlation between infestation and yield.

Regression equations: trial A, $y = 45{\cdot}6 - 0{\cdot}5x$; B, $y = 47{\cdot}6 - 0{\cdot}69x$; C_1, $y = 48{\cdot}6 - 0{\cdot}435x$; C_2, $y = 39{\cdot}3 - 0{\cdot}415x$; D (mean), $y = 34{\cdot}5 - 0{\cdot}48x$; D_4, $y = 66{\cdot}5 - 0{\cdot}87x$; D_6, $y = 52{\cdot}8 - 0{\cdot}45x$; E, $y = 14{\cdot}55 - 0{\cdot}23x$.

DISCUSSION

Within the limits of the infestation rates recorded, namely, 0–50%, there is little doubt that the relation between yields and infestation is rectilinear. The best fitting lines, those of trials B and C, are straight regression lines.

When an attempt is made to arrive at a general line for the regression of yield on infestation, it becomes obvious that the higher yielding trials A, B, C and D can be grouped together around a common regression line with slope about $-0{\cdot}5$, while trial E stands apart with a slope about $-0{\cdot}2$.

In testing for homogeneity about a common line for the high-yielding group, trial D has been omitted as correlation is poor for the reasons stated. The analysis has been carried out on the differences of the actual infestation and yield figures from the means for the respective trials, thus bringing the means of the lines together at a common mean on the generalized regression line. For instance, in trial A, with a mean $x = 13$, mean $y = 38$, a pair of observations such as $x = +7$, $y = +38$, becomes $x = -6$, $y = 0$, and similarly for other pairs of observations.

The grouped correlation coefficient is found to be $-0{\cdot}844$ for 62 D.F., P is greater than 0·001, and there is thus high significant correlation about a common regression line of slope $b = -0{\cdot}546$ and 5% confidence limits of $\pm 0{\cdot}088$. This represents the effect of infestations up to about 50% on crops with a mean maximum yield of 4100 lb./acre. It can also be expressed as a generalized regression line $y = 45{\cdot}1 - 0{\cdot}55x$.

Regarding trial E, with a regression line of slope about $-0{\cdot}2$, the 5% confidence limits are $\pm 0{\cdot}101$, indicating that the regression lines for high- and low-yielding groups are distinct. When the two regression coefficients are compared by a t-test

of the differences between their variances, however, $t = 1{\cdot}94$ for 65 D.F., which very nearly reaches the 5% level of significance ($t = 2{\cdot}00$).

The proposition that the regression lines of high- and low-yielding groups are different is supported by the arrangement of all the lines when drawn and also by the regression lines for the two blocks of trial D, which have slopes proportional to their maximum yields. This relationship between slope and maximum potential yield can be stated as $b \propto \bar{y}$, and in Fig. 2 these figures are plotted, showing how the slope increases with increasing maximum yield. The same relationship has been found for the effect of *Pyrausta* on yield by Deay *et al.* (1949) and his figures have been extracted and plotted in Fig. 3 for comparison.

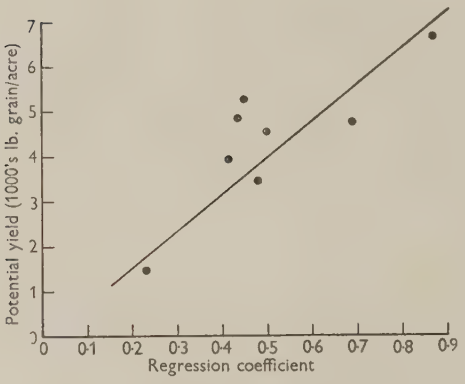

Fig. 2

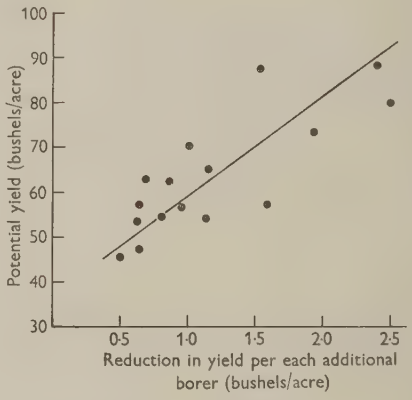

Fig. 3

Fig. 2. The relation between the regression coefficient, b, and the potential yield of grain in the absence of borer.

Fig. 3. The relation between the reduction in yield per each additional borer per plant and the potential yield of grain in the absence of borer (based on figures given by Deay *et al.* 1949).

There is an alternative model for the arrangement of a family of regression lines, namely, that the lines might be parallel and the reduction in yields for the same increase in infestation be absolute, and not proportional. If this were so the use of a yield factor to express an increase in yield over controls would be unreliable as an indication of the change in infestation. It would also imply that the same degree of control by insecticides would give the same increase in yield on different crops. The evidence above is against this, and supports a model in which regression lines converge.

A regression line will not necessarily meet the x-axis at 100% infestation. This would occur if an infested plant invariably yielded nothing. In practice, an infested plant usually suffers severely, depending on the number of larvae infesting it and its age when attacked, but it may produce a small yield which would be sufficient to give a small crop from a plot which is 100% infested, particularly if infested late.

Alternatively, the complete regression line may be curvilinear, but rectilinear over the part which is of practical concern.

The meaning of the regression equations can be stated in terms of the amount of increase in yield per percentage reduction in infestation. For the higher yielding group this is about 35 lb. of grain for every 1% of plants infested. For the lower yielding group, the figure is about 17 lb. of grain for every 1%. On account of the transformation of the percentages to angles for analysis, the figures given vary over the range of untransformed percentages, and those given are averages over a range of 10–70% for the higher yielding group, and 10–30% for the lower.

Application

The establishment of a relationship between infestation and yield and its expression as a regression equation of known value is helpful for understanding the mechanism of damage by the insects concerned. Final yields are the result of a number of growth processes which may be inhibited, retarded or encouraged, directly or indirectly, by the pest. If the graphic relation is rectilinear, plant destruction is proportional and straightforward, but if the yield/infestation curve falls rapidly at first, or more rapidly later, or even rises and then falls as infestation increases, as may occur in the case of *Lygus* on cotton (Geering, 1954), there is a suggestion as to how damage is being done.

The relationship can be used to calculate the level of infestation which can be tolerated in order to obtain an economic yield. If the same degree of control is obtained, at the same cost for insecticide, but the yield increase depends on the potential yield of the crop, high-yielding crops will give a greater return for the same outlay on insecticides. For some very low-yielding maize crops of peasant Africans the increased yield may not be worth the expense of insecticide.

Similarly, in sampling for infestation, the limits of error of a sample are equivalent to a greater weight of yield for crops of high potential yield. If the slope of the regression line is less, less rigid limits of error would suffice, in terms of the expected yield or its value. By this means a method of infestation sampling can be chosen which gives an adequate limit of accurcy in relation to the labour, time and expense of taking it.

A stimulus is also given to a closer investigation of the time at which damage is critical, and of the dynamics of the insect population during the course of the infestation.

The validity of a 'yield factor' for comparing the differential effects of insecticide treatments depends on the way regression changes for crops of different maximum expected yields. A yield factor, which expresses the number of times by which the yield resulting from an insecticide treatment exceeds that obtained from an uncontrolled infestation, has been used to compare the effects of insecticide treatments (Walker, 1960). It will be seen that it is only misleading as a statistic if the regression lines are related in a different way than appears to be the case.

It must be stressed that these results are estimates of the relationship between infestation and yield at particular places under particular conditions. They should

not be taken as the true relationship under wider conditions without further investigation. In addition, routine control treatments in practice are sometimes not as effective as experimental applications (Strickland, 1957). There is also the danger of accepting yields from small plots as a reliable measure of the yields from adjacent fields. More information about the relation between yield and infestation would result from a study of the change in regression as infestation occurred at different times in the life of the plant. It would be expected that the regression coefficient would become less as infestation occurred later, irrespective of more or less significant correlation at different phases in plant growth.

No true understanding of the relationship is possible until the partial correlation coefficients can be analysed between yield and stem-borer and the other major factors that depress yield, such as drought, lack of soil fertility, rust, termites or other insects.

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THE ACTION OF COPPER IN ANTIFOULING PAINTS

By D. J. CRISP and A. P. AUSTIN

Marine Biology Station, Menai Bridge, Anglesey

(With 5 Text-figures)

Experiments on the number of barnacle cyprids found on plane surfaces and on the surface of small pits coated with compositions containing different amounts of cuprous oxide show that the presence of copper does not deter the cyprids from attempting to settle. Copper-oxide paints do not inhibit settlement appreciably; their efficiency as antifouling agents is caused by the toxic action of copper on cyprids and young spat after initial attachment.

The cementing of the shell to the substratum in young barnacles which have survived metamorphosis appears to be hindered by the presence of copper paints so that they are easily dislodged.

Introduction

Cuprous oxide has for long been used as the main toxic constituent of antifouling paints, the efficiency of which in this respect is known to be related to the rate of leaching of copper ions into the water adjacent to the paint film. The toxic properties of copper toward most marine fouling organisms are also well attested. It is therefore certain that if a larva settles on a paint film from which copper is being leached at a sufficient rate, the larva will be poisoned, drop off, and the surface will thus be kept clean. However, it is now known that, when the larvae of many marine organisms are about to settle, they explore the surface with which they are in contact and attach only if it is attractive enough to stimulate fixation. It is therefore possible that a paint might liberate a concentration of copper which was not sufficient to poison, but which might be sufficient to interfere with the pre-settlement behaviour pattern of the organism. Were this so, the toxic action of copper would be incidental to its primarily deterrent action. The alternative is that the presence of copper ions might have no influence on initial settlement, and the antifouling action be due entirely to post-settlement toxicity. It is at first surprising that these alternatives have not been experimentally investigated and distinguished; the reason is that insufficient attention has been given to the behaviour of larvae during the pre-settlement phase. Antifouling action has hitherto been investigated by comparing the amount of successful settlement on toxic and control surfaces. Often in the course of testing antifouling paints numbers of partially or fully metamorphosed cyprids, which later drop off, have been observed on the toxic surfaces. This fact indicates that any deterrent action which copper may possess is incomplete and is less important than its toxic action.

In the experiments described in this paper the possible influence of copper ions on the settlement process is investigated.

Settlement on plane surfaces of different toxicities

Balanus balanoides (L.) does not settle readily on a plane surface; the results obtained on such surfaces using this species were therefore rather indefinite owing to the low rates of settlement.

A number of panels of laminate phenol-formaldehyde plastic ('Tufnol') measuring 20 × 27 cm. were uniformly roughened by rubbing against a sheet of glass with an aqueous paste of fine carborundum. On each, nine areas of 9 × 2·5 cm. were marked out and painted with one of three paints, designated α, β and γ, in the form of a latin square, each paint occurring once in each row and in each column. The area of unpainted control surface left was equal to twice the area of each of the three paints.

Paint α, which was strongly toxic, contained 40% of cuprous oxide dispersed in a vehicle consisting of 87·5% of rosin and 12·5% ester gum. Paint β contained the same amount of cuprous oxide as paint α but was compounded with a vehicle consisting of 60% of rosin and 40% of ester gum and was therefore less soluble in sea water. Paint α leached copper at a rate slightly in excess of 10×10^{-6} g./cm.^{2}/day and paint β at approximately 5×10^{-6} g./cm.^{2}/day. Paint γ was a non-toxic counterpart of paint β containing 48% of Turkey red oxide in a vehicle consisting of 60% rosin, 40% ester gum. The surfaces were exposed in a vertical position in the Menai Straits, one set at approximately the level of mean low water of spring tides in a shaded position on the piles of St Georges' Pier, and another set totally immersed from beneath the pontoons. In both positions the panels were exposed to a good flow of sea water. In the former position the main settling organism was at first *B. balanoides*, but after May this species gave place to *Elminius modestus* Darwin. No obvious differences were evident between panels, between exposure sites, or between species. In view of the rather small numbers settling, the results from both positions have been grouped in Table 1.

Table 1. *Settlement of barnacles on panels immersed on* 12. v. 58

	Paint α		Paint β		Paint γ		Rough plastic	
Date	Total	Cyprids	Total	Cyprids	Total	Cyprids	Total	Cyprids
15. v.	0	0	6	5	5	5	106	83
17. v.	5	4	4	3	5	3	167	111
19. v.	3	2	7	0	24	9	288	40
20. v.	4	2	11	3	33	10	364	41
21. v.	4	0	15	4	46	13	—	39
26. v.	1	0	14	3	52	11	394	25
3. vi.	1	0	15	1	156	38	337	7
15. vii	48	14	57	19	3527	53	818	31

It is clear that none of the painted surfaces was initially as attractive as the rough plastic; probably the cyprids of *Balanus balanoides*, the species which predominated at the outset, chose the more roughened surface; this species is known to settle preferentially on roughened surfaces (Barnes, 1956). Later in the experiment,

when *Elminius* cyprids predominated, the numbers on the smooth non-toxic paint overtook the numbers on the rough plastic. This result was partly due to the greater tolerance of *Elminius* cyprids for smooth painted surfaces and partly to the fact that the previously settled barnacles had grown to occupy much of the space and so excluded the heavy spatfall of *Elminius* from the rougher surface.

If we consider only the settlement on the three painted surfaces, where surface texture was similar, it will be seen (Table 1) that during the first few days settlement occurred on all the paints and that the numbers settling were of a similar order of magnitude. After metamorphosis, however, few barnacles remained alive on paint β and fewer still on paint α. However, on the non-toxic paint γ the number steadily grew and the numbers of cyprids alighting also increased. This was probably due to the persistence of previously settled spat which stimulates further settlement and so leads to an increasing rate of settlement (Knight-Jones & Crisp, 1953). On the toxic paints the cyprids and recently metamorphosed individuals either died or failed to attach properly and were soon washed off in the sea. There would therefore be little gregarious stimulation of cyprids to settle on these areas and the numbers of cyprids accordingly showed no comparable increase. After July a small number of spat metamorphosed and remained alive for a few days and larger numbers of cyprids were then found on the surface.

Owing to the very small numbers settling on plane surfaces, it was not possible to be sure whether the slightly smaller numbers that settled initially on the toxic paints α and β, compared with the numbers on the non-toxic paint γ, indicated that some were washed off or that fewer attempted to settle. The initial repellent action, if any, cannot however be very marked.

Settlement in pits coated with toxic and non-toxic paints

Small pits in a surface are highly attractive to cyprid larvae (Crisp & Barnes, 1954) and can be used greatly to increase the amount of settlement taking place initially on the panels. Treated with paints of varying toxicity, the influence of the paint on the attractiveness of the pits may easily be measured from its effects on the rate of settlement.

Preliminary experiments showed that not all pits were equally attractive. The pits became more attractive, in terms of numbers settling per unit area, as the radius of curvature decreased down to a radius approximately equal to half the length of the cyprid. The larger pits, though less attractive in terms of numbers per unit area, were, nevertheless, capable of accommodating a greater number of settled larvae before they were filled up. The important feature of the experiment was the possibility of using pits of varying degrees of attractiveness to the larvae. Three sizes were chosen, having a radius of curvature 1·85 mm. (size 1), 1·26 mm. (size 2) and 0·53 mm. (size 3). These pits, but not the surrounding plane areas, were painted in equal numbers with the paints α, β and γ. The pattern in which the pits were arranged was found to be of considerable importance. Most larvae reach the pits by crawling over the surface; moreover, they tend to crawl upwards on

panels exposed vertically. Hence if the pits are close together the outer ones are colonized first, especially the uppermost. To give all combinations of pits and paints equal opportunity of settlement, the pits could, in theory, be widely separated from one another, but unfortunately it is practically impossible to attain uniform settlement over a large area. The alternative was to place the pits close together (1 cm. between centres) in three square patterns of 6×6. The arrangement was so designed that if the paints were compared one with another in pairs, each pair of paints (β, γ, or α, γ) and each size of pit (1, 2, 3) was present once in each vertical and once in each horizontal row, and also was represented equally in the four outer corner pits (A, Fig. 1), in the remainder of the twenty outer pits of the squares (B, Fig. 1) in the next row (C) and in the inner pits of the squares (D). Such an arrangement was possible with three 6×6 latin squares. The full arrangement involving paints α and γ is shown in Fig. 2.

A	B	B	B	B	A
B	C	C	C	C	B
B	C	D	D	C	B
B	C	D	D	C	B
B	C	C	C	C	B
A	B	B	B	B	A

Fig. 1. Four types of position in a 6×6 square with different accessibility to exploring larvae. A, outer corners; B, outer edges; C, intermediate positions; D, inner positions.

The pits were drilled out on panels measuring 20×27 cm. using dentist's drills to obtain a smooth hole without any lip, and painted thinly with the appropriate pair of paints (α and γ or β and γ). The panels were placed in the sea at and just above mean low water of spring tides on St George's Pier, Menai Bridge, and removed at intervals for examination. Readings both of the number of metamorphosed and recently settled cyprids were taken at first daily. As the pits filled up the rate of settlement slowed down and the interval was accordingly increased.

The results for total numbers settled in two pairs of experiments in which paints α and γ and β and γ were compared are shown in Figs. 3 and 4. The settlement was very much greater than on plane surfaces, whether the pits had toxic or non-toxic paint covering them. Fig. 3 shows how the number of barnacles per pit increased throughout the experiment, counting both metamorphosed and unmetamorphosed individuals. Many of those in pits coated with paint α and β were dead but remained attached and so were included. Although the number of barnacle spat was in general greatest in the non-toxic pits γ, and less in the α and β pits, the differences

were not large. Moreover, instead of the total number settled, a comparison can be made of the number of unmetamorphosed cyprids observed daily throughout the experiment. Since the cyprids metamorphose in about 24 hr., this corresponds

1α	1γ	3α	2α	3γ	2γ
3α	1α	1γ	3γ	2γ	2α
1γ	3α	1α	2γ	2α	3γ
3γ	2γ	2α	3α	1α	1γ
2γ	2α	3γ	1γ	3α	1α
2α	3γ	2γ	1α	1γ	3α

3α	1α	2γ	2α	3γ	1γ
1α	3γ	1γ	2γ	2α	3α
3γ	3α	2α	1γ	2γ	1α
1γ	2α	3α	3γ	1α	2γ
2γ	1γ	3γ	1α	3α	2α
2α	2γ	1α	3α	1γ	3γ

3γ	2γ	2α	3α	1α	1γ
2α	3γ	2γ	1α	1γ	3α
3α	1α	1γ	3γ	2γ	2α
1γ	3α	1α	2γ	2α	3γ
2γ	2α	3γ	1γ	3α	1α
1α	1γ	3α	2α	3γ	2γ

Fig. 2. Arrangement of the three sizes 1, 2 and 3 and the two paints α and γ in three 6 × 6 squares designed to equalize all combinations of pits and paints both in the four possible positions A, B, C and D, illustrated in Fig. 1, and in all rows and columns of each square.

approximately to the daily rate of settlement. The numbers of cyprids, though similar, were in fact greater for the pits coated with toxic paint than for pits coated with non-toxic paint (Table 2). The slightly lower settlement in the toxic pits is therefore due, not to fewer cyprids attaching, but to greater mortality and subsequent loss of cyprids or barnacles from these pits.

The daily rate of settlement, determined by the cyprid count at the end of the day, rose at first as the pits began to be occupied. This, as in the experiment using plane surfaces, may be attributed to the gregarious tendency (Knight-Jones & Crisp, 1953). The subsequent levelling off in the number of cyprids in the painted

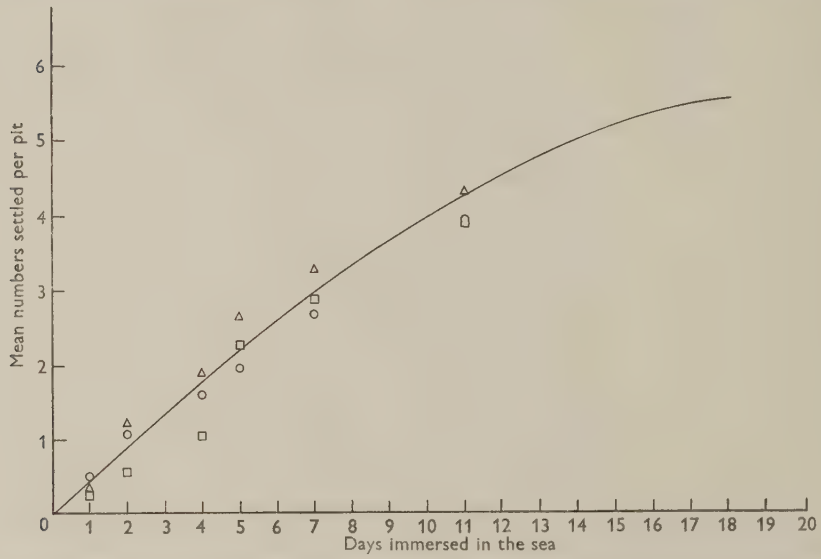

Fig. 3. Number of cypris larvae, averaged over all sizes of pit for each of three paints coating the surfaces of the pits, plotted against the time of exposure of the pits to barnacle settlement; □ = α, most toxic with a leaching rate of copper in excess of 10×10^{-6} g./cm.²/day; ○ = β, moderately toxic with a leaching rate of 5×10^{-6} g./cm.²/day; and △ = γ, non-toxic paint with otherwise similar composition to β.

pits after about 10 days can be ascribed to the pits having become so crowded that insufficient space was left for many more to settle. In the γ series of non-toxic pits, where most of the settled individuals survived and did not fall off, the daily rate of settlement dropped abruptly after 10 days.

The results for the pits painted with each of three sets of paints were pooled to arrive at a mean value of settlement in each size of pit (Fig. 4). This settlement is clearly different for the three kinds of pits.

Initially the number settling in all three sizes of pits 1, 2 and 3 was about equal. Since the smallest pits, those of radius 0·53 mm., have an area of less than one-eighth that of the largest pits (radius 1·85 mm.), the number settling per unit area

was much higher. With increasing numbers settling, however, the larger pits became more crowded so that by the end of the experiment the number settled per unit area (1 mm.^{2}) in the largest pits was 0·768 compared with 1·67 in the smallest pits.

If the difference between the numbers settled in toxic and in non-toxic pits were

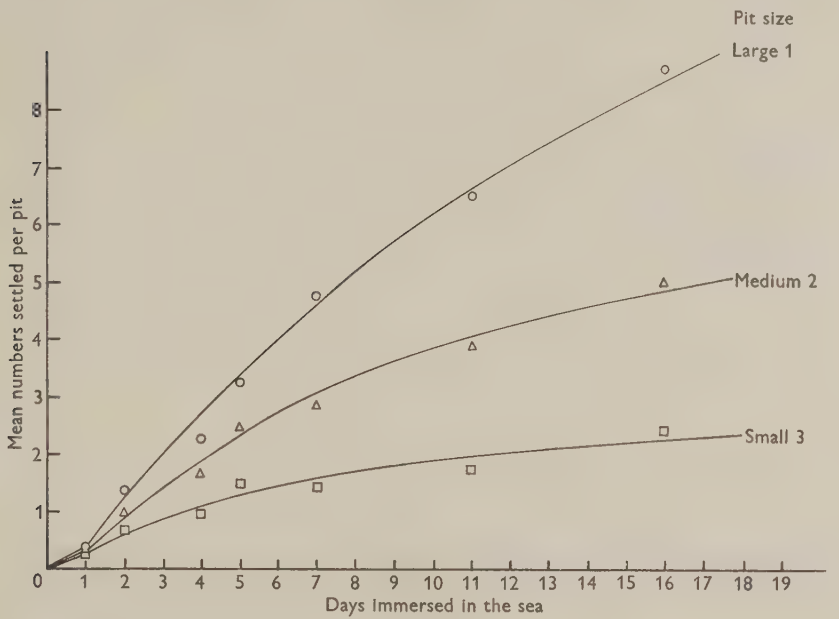

Fig. 4. Number of cypris larvae averaged over all types of paint for each of the three sizes of approximately hemispherical pits; large no. 1, r = 1·85 mm., area 23·7 mm.^{2}; medium no. 2, r = 1·26 mm., area 8·7 mm.^{2}; small no. 3, r = 0·53 mm., area 2·8 mm^2.

TABLE 2. *Average number of cyprids observed per pit*

Interval of time from 26. iv. 59 (days)	Pits coated with paint		
	α	β	γ
	0	0	0
2	0·22	0·47	0·31
3	0·44	0·80	1·00
4	0·83	0·94	1·18
5	1·77	1·45	1·43
6	1·80	0·87	1·05
8	2·67	1·51	1·72
10	1·87	1·76	2·48
12	3·35	2·26	0·90
15	1·77	2·14	0·58
Total	14·72	12·20	10·15
No. of metamorphosed barnacles on 15th day	3·16	3·81	5·21

due to a differential mortality, then during the first few days this difference should be small, but should later become increasingly significant as the barnacles in the pits died off. To test this the ratio of the mean numbers, (n_α, n_β), in the toxic pits α and β, to the number per pit settled on those covered with non-toxic paint γ, (n_γ), was expressed as a logarithm. For equal settlement in toxic and non-toxic pits the value should approach zero. Six sets of such ratios were available for each of the first 3 days of the experiment and an analysis of variance was done to find the value of the standard error associated with the mean values of log (n_γ/n_α) and of log (n_γ/n_β). A mean value of either which significantly exceeded zero would imply that the number settled on the toxic surface was reduced below that of the control. Table 3 gives the actual mean ratios of numbers settled, asterisks indicating values significantly greater than unity at the 0·05 probability level. There was little difference in the settlement on the first day, but later, as the effect of mortality became felt, the total numbers in the toxic pits became reduced.

Table 3. *Ratio of number settled on control paint γ to number settled on toxic paint (α or β); asterisk indicates ratios significantly in excess of unity*

	Comparison	
Time	n_γ/n_α	n_γ/n_β
1st day	1·35	1·01
2nd day	2·04*	1·41*
3rd day	1·55*	1·55*

Further experiments confirmed that settlement took place in both toxic and non-toxic pits from the very first day of immersion. Where the settlement was heavy enough for the numbers to be significant, the settlement in the non-toxic pits exceeded that in the toxic pits even on the first day. The total numbers of cyprids observed to attach during the whole course of the experiment was also greater in the non-toxic pits, but, however, the difference was not significant.

Influence of toxicity of surrounding surface on settlement in pits

It is well known that cyprids explore a surface thoroughly before fixation. A typical set of tracks over a grooved surface is shown in Fig. 5. It can be seen that just before attachment the cyprid makes a very complete exploration, moving freely in and out of grooves or pits.

To test whether there was any tendency for these exploring cyprids to leave a toxic surface before fixation, an experiment was designed taking advantage of the fact that the cyprids will normally walk over the area surrounding each pit before entering it. If, therefore, this area were coated with toxic paint and the cyprids were thereby encouraged to swim off, such pits should be slower to acquire settlement. If, on the other hand, the cyprids walked across the toxic area with impunity, these pits should be occupied just as much as others with a non-toxic surface surrounding them.

Four types of similar sized pits 8·7 mm² in area and 1 cm. apart, W, X, Y, Z, each coated with, or surrounded by, different paints, were therefore offered to the cyprids. A rosin-based cuprous oxide paint known to be effective was used to provide a standard toxic coating. In type W both the pits and the panel surface were coated with toxic paint; in type X only the pit was painted. In type Y the surface surrounding the pit was painted while the pit was left clean, whilst in type Z both the surface and the pit were left clean.

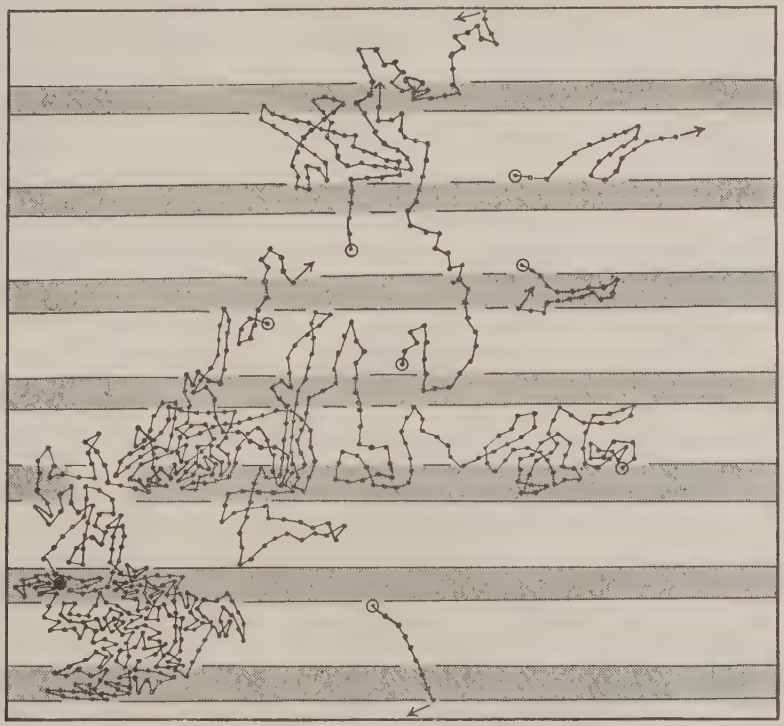

Fig. 5. Paths traced by means of camera lucida, of seven cypris larvae exploring a grooved non-toxic panel. The grooves are shaded and approximately 5 mm. apart. The point of initial attachment is shown by an open circle, the point of swimming off by an arrow. One cyprid, which explored for 30 or 40 minutes, eventually settled and metamorphosed in the groove at the point marked with a full circle.

In the first experiment twelve horizontal rows of twenty pits of each type were set out and exposed to settlement at low-water mark as previously described. Greater settlement took place in the uppermost rows due to the tendency of cyprids to climb the panel (cf. Crisp & Barnes, 1954). This effect was taken into account in an analysis of variance and shown to be significant (Table 4). After 4 days the numbers settled in W, X, Y and Z type pits did not show any significant difference,

the totals being 54, 44 and 59, respectively. The analysis was repeated for the results obtained on subsequent examinations after 8, 16 and 25 days with the same result (Table 4).

TABLE 4. *Analysis of variance of settlement in pits of types W, X, Y and Z*

W, toxic pit and surround; X, toxic pit, non-toxic surround; Y, non-toxic pit, toxic surround; Z, non-toxic pit, non-toxic surround.

Source of variation	Variance ratios (relative to error variance) for results taken after				Significance value for variance ratio at $P = 0{\cdot}05$
	4 days	8 days	16 days	25 days	
Experiment 1. Treatments arranged horizontally, immersed 3. v. 57					
Between treatments W, X, Y and Z	2·4	0·7	1·6	0·1	4·76
Between horizontal rows of pits	17·4*	12·9*	18·1*	14·4*	5·14
Experiment 2. Treatments arranged vertically, immersed 9. v. 57					
	2 days	7 days	14 days	—	
Between treatments W, X, Y and Z	1·38	0·74	0·93	—	4·76
Between vertical rows of pits	3·95	5·70*	3·60	—	5·14

* Significantly greater than error variance.

In the second experiment, the four types of pit, W, X, Y and Z, were placed in vertical rows; little gradation in settlement was noticed between the rows, so each of the three rows of a given treatment, W, X, Y, Z, etc., could be regarded as a replicate. A simple analysis of variance on the settlement numbers after 2, 7 and 14 days again showed no significant differences due to treatment and the differences between the rows were greatly reduced and scarcely significant (Table 4). There was, however, a tendency for the unpainted pits Z to be slightly superior numerically, as indeed would be expected on account of losses through mortality in W, X and Y.

A further experiment, in which the combinations W, X, Y and Z were arranged in latin square designs, gave similar results, but without any significant positional effects. The initial settlements were about equal in all four types of pit, but the final numbers were greater in Y and Z than in W and X on account of differential mortality (Table 5).

TABLE 5. *Total numbers settled in* 64 *pits equally distributed among types W, X, Y and Z* (*see Table* 4) *as four latin squares* (*immersed* 7. v. 57)

Time interval (days)	Type of treatment			
	W	X	Y	Z
5	12	6	12	8
8	26	18	23	32
13	38	37	48	46
25	44	38	53	54

Influence of toxic surfaces after settlement

A further series of experiments was carried out similar to those just described using treatments W, X, Y and Z on equal numbers of pits. The panels were kept for a longer period, however, and photographs taken at intervals to follow the changes that occurred after settlement. The paint used was of marginal toxicity, thus allowing some of the barnacles to metamorphose and continue growing in the presence of the paint.

In the first experiment approximately equal numbers settled initially on the pits W, X and Y, while slightly more settled in Z, the untreated pits. However, the numbers which metamorphosed and survived showed much greater differences, the pits containing paint (pits W and X) causing high mortality, as shown in Table 6. In the pits which were merely surrounded by toxic paint (Y) the number settled was similar but the number metamorphosed much smaller than in the control pits Z (Table 6).

Table 6. *Influence of copper-painted surfaces on settlement and subsequent metamorphosis. The pits containing copper paints, W and X, show similar settlement but greater mortality (immersed 7. v. 57)*

	Pit type			
	W	X	Y	Z
	Total settled per pit			
After 3 days	0·10	0·08	0·17	0·22
After 6 days	0·27	0·19	0·26	0·31
After 14 days	0·75	1·10	1·10	1·30
	Total metamorphosed			
After 3 days	0·01	0·01	0·04	0·05
After 14 days	0·20	0·27	0·63	1·03

In the second experiment, carried out when the settlement of *B. balanoides* was almost over, the pattern of settlement was similar, the number found in the pits W, Y and Z being roughly equal, though in the X pits rather fewer settled than was expected. Possibly on this occasion too much paint was applied, causing the contour of some of the pits to be altered or obscured. In Table 7 the numbers settled in the W, X and Y type pits are expressed as fractions of the numbers settling in the control pits Z. It can be seen from total settlement that there was a tendency for the numbers in the toxic pits relative to those present in the non-toxic controls to diminish with the passage of time. The details of size distribution given against each date point to the fact that in the toxic pits W and X, and to a lesser degree in the non-toxic pits Y (which were surrounded by a toxic surface), the older groups in the population become eliminated to a much greater degree than in the control pits; whereas about a third of the cyprids metamorphosed on the toxic surface, none reached a size exceeding 3 mm. Evidently a paint with a critical leaching rate of copper kills barnacles that are just metamorphosing but also continues slowly

to kill off those which have survived metamorphosis and are growing to a diameter of 2–3 mm. This result suggests that the metamorphosing cyprid is little, if any, more sensitive to copper than the recently metamorphosed spat. The reason, therefore, that during the settlement period the total numbers are maintained in pits

TABLE 7. *Influence of copper-painted surfaces on size distribution of settled barnacles* (*immersed* 18. v. 57)

Time exposed		Pit type W	X	Y	Z
2 days	Total settlement	0·86	0·57	0·96	1·00
	Metamorphosed only	0·24	0·27	0·57	1·00
8 days	Total settlement	0·71	0·53	0·97	1·00
	1·2 mm. (*c.* 6 days old)	0·22	0·28	0·39	1·00
24 days	Total settlement	0·51	0·37	0·89	1·00
	Metamorphosed	0·42	0·34	0·85	1·00
	1·2 mm. (*c.* 6 days old)	0·27	0·21	0·61	1·00
	2·0 mm. (*c.* 14 days old	0·05	0·06	0·26	1·00
	2·0 mm. (*c.* 14 days old)	0·05	0·06	0·26	1·00
	3·0 mm. (*c.* 21 days old)	0·00	0·00	0·03	1·00

The entries are the ratios or numbers in the toxic pits (W, X and Y) compared with the number in non-toxic (Z) pits taken as unity. Fewer barnacles survive to larger size in toxic pits.

coated with such paint at a density roughly equal to that in the control pits is that more cyprids attach as fast as those that are killed fall off. Thus the paint is effective only because it causes mortality and not because it causes any reduction in the rate of initial attachment. The small barnacles that survive metamorphosis do not appear to be properly attached to the paint film and can very readily be dislodged in comparison with spat of similar size growing on an inert surface. The bases of these unhealthy spat are swollen and convex toward the paint surface, often being attached by the antennulary cups only. The basal parts of the calcareous parietes are often distorted and do not spread over or attach firmly to the surface as in normally growing barnacles.

CONCLUSIONS

The tendency of cyprids to settle in small pits was scarcely influenced by the presence of paint films with a well-established antifouling action due to release of copper. Even at the outset, when there had been little or no loss of cuprous oxide by leaching, the number of cyprids that attempted to settle in pits painted with a toxic film was of the same order as the number that settled in pits painted with a non-toxic but otherwise similar paint. When the area around the pit was painted with a toxic paint, the pits were occupied to much the same extent as they were when the surface around them was chemically inert. All these facts suggest that barnacle cyprids are insensitive to copper ions during the period that they are searching for sites on which to settle. On plane surfaces, however, where the settlement was poor, a rather greater difference was observed between toxic and non-toxic areas (Table 1). Probably the poorly attached and unhealthy cyprids

were washed off much more readily from the plane toxic surfaces than from the toxic pits. The cavities of the pits would be protected slightly from the shearing action of waves as they washed across the surface of the panel. It is also possible that the concentration of copper adjacent to a large flat surface of paint would exceed that produced by the leaching of the small spot of paint within each pit; if so a larger proportion of cyprids might have been killed before becoming cemented to the plane surface.

When the more suitable surrounding areas of rough plastic had been colonized many more spat began to settle on the non-toxic paint. They settled for the most part near the heavily colonized areas of rough plastic, strongly suggesting that the gregarious stimulus to settlement had overcome the normal avoidance of a smooth surface. Again, when the non-toxic paint was well colonized more attempted to settle nearby on the toxic paints and indeed a few succeeded. These observations are thus similar to those of Harris & Forbes (1946) and Stubbings (1957), who referred to this as the 'reversed border' effect. We consider from the above results that this effect is mainly caused by gregarious settlement (Knight-Jones, 1953).

The results as a whole, both on the plane and pitted surfaces, show that, while a reduction in the number of cyprids attaching may be caused by the leaching out of copper, such repellent action as may exist is a very minor factor in the action of the paint. It is readily overcome by the propensity of the cypris larvae to settle in pits. The effective antifouling action is therefore entirely due to the toxicity of the paint, which slowly kills off the small barnacles during and often after metamorphosis. In those which survive it interferes with the attachment of the base of the barnacle to the paint film so that they can readily be washed off.

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THE INFLUENCE OF GROWTH STAGE ON THE RESPONSE OF RED CLOVER (*TRIFOLIUM PRATENSE* L.) TO GIBBERELLIC ACID

By J. L. STODDART

Welsh Plant Breeding Station, Aberystwyth

(With Plate 14 and 1 Text-figure)

The response of seedling and first harvest year plants of red clover (*Trifolium pratense* L.) to treatment with gibberellic acid (GA) at various growth stages is described.

Seedlings sprayed before the seventh leaf stage developed into single-stemmed plants; treatment with GA at the third- or fourth-tiller stage resulted in final stem numbers similar to those of controls. Emergence was earliest, and the number of heads per plant greatest where sprayings were delayed until the third- or fourth-tiller stage.

In first harvest year plants significant increases in the number of heads per stem were obtained with certain treatments, especially those which had two applications of 0·5 mg. GA per plant during the elongation of the first four internodes. This was related in all treatment groups to modifications of the branching pattern, and also to the increased incidence of multiple heading. Earlier emergence resulted from treatment at all growth stages, the effect being maximal where three well-spaced sprayings were applied during active stem extension.

Introduction

Stoddart (1959) showed that late-flowering red clover (*Trifolium pratense* L.) responded readily to applied gibberellic acid (GA) and although many striking effects were noted subsequent attention was centred mainly upon those influencing flower production.

The shortening of the interval between floral initiation and flower emergence due to the more rapid extension of stem internodes, suggested the possibility of obtaining seed from late-flowering red clover crops in the year of sowing. Furthermore, the GA-induced modifications of branching, and the attendant increase in the number of heads per plant, offered the prospect of greater seed yields if the response could be reproduced on a field scale.

The initial studies were based upon an arbitrary treatment-pattern orientated to time rather than to growth stage, and although such a system of treatment is valid with clonal material under controlled conditions, it may not be applicable to populations exposed to climatic variables. It was decided to attempt to define the stages in the growth cycle at which applied GA would give the optimum response.

Material and methods

The Station-bred variety of extra-late-flowering red clover, Aberystwyth S.123, was used. Owing to the number of plants involved the experiment was divided between 2 years. In the first year a study was made of the response of seedling and first harvest year plants to a single spraying of GA and in the second year, the effects of double and triple sprayings on mature plants.

Seedlings were grown in shallow boxes from seed sown in April 1958, and thinned to a density of six plants per box before use. Mature plants were clonally propagated from a single plant, the propagants being split and planted into 7 in. pots during early April in 1958 and 1959.

A light loam was the universal growth medium and no manures were applied. In the early stages of growth both pots and boxes were watered to capacity three times per week, but during the active growth period in May and June daily watering was necessary.

All plants were kept in an unheated glasshouse throughout the experiment.

Treatments

For seedlings the dosage of GA was standardized at 0·1 mg. per plant applied as a 10 ml. aqueous foliar spray. First harvest year plants received 0·5 mg. per plant per application in a spray volume of 50 ml.

In the early stages of growth seedlings were sprayed in relation to the number of trifoliate leaves produced, and later to the number of leaf buds, or 'tillers', forming the rosette. The treatments are summarized below

Treatments	Abbreviations
Control—untreated	Control
Sprayed at 3rd trifoliate leaf stage	3L
Sprayed at 7th trifoliate leaf stage	7L
Sprayed at 9th trifoliate leaf stage	9L
Sprayed at 3rd tiller stage	3T
Sprayed at 4th tiller stage	4T

First harvest year plants were treated at stages coinciding with the elongation of various internodes, i.e. when sprayed, the extended internodes visible in the most advanced stem of the plant were used to indicate the growth stage.

Sprayings corresponding with the elongation of the first internode and applied in mid-April are termed 'vegetative phase' treatments.

The critical daylength for flowering in S. 123 is reached in mid-May but internode elongation commences before this date. Daylength becomes critical at about the time the second internode is completed, and from this point onwards it must be assumed that floral induction has taken place, in spite of the fact that morphological changes at the shoot apex do not become visible until the sixth or seventh internode stage.

The treatments applied to first harvest year plants were as follows

Years	Treatments	Abbreviations
1958	Control	Control
	Sprayed once in 'vegetative phase'	V
	Sprayed once at 2nd internode stage	2
	Sprayed once at 4th internode stage	4
	Sprayed once at 8th internode stage	8
1959	Control	Control
	Sprayed in 'vegetative phase' and at 2nd internode	V+2
	Sprayed in 'vegetative phase' and at 4th internode	V+4
	Sprayed in 'vegetative phase' and at 8th internode	V+8
	Sprayed in 'vegetative phase' and at 10th internode	V+10
	Sprayed at 2nd and 4th internode	2+4
	Sprayed at 2nd and 8th internode	2+8
	Sprayed at 2nd and 10th internode	2+10
	Sprayed at 4th and 8th internode	4+8
	Sprayed at 4th and 10th internode	4+10
	Sprayed in 'vegetative phase' and at 2nd and 4th internode	V+2+4
	Sprayed in 'vegetative phase' and at 2nd and 8th internode	V+2+8
	Sprayed in 'vegetative phase' and at 2nd and 10th internode	V+2+10
	Sprayed in 'vegetative phase' and at 4th and 8th internode	V+4+8
	Sprayed in 'vegetative phase' and at 4th and 10th internode	V+4+10
	Sprayed in 'vegetative phase' and at 8th and 10th internode	V+8+10
	Sprayed at 2nd, 4th and 8th internode	2+4+8
	Sprayed at 2nd, 4th and 10th internode	2+4+10
	Sprayed at 2nd, 8th and 10th internode	2+8+10
	Sprayed at 4th, 8th and 10th internode	4+8+10

These abbreviations will be used throughout this paper.

Replication and lay-out

The seedling treatments were applied on a box basis, there being four replicates of each treatment and six plants per box, giving twenty-four plants to be sprayed at each growth stage. A 6×4 randomized block lay-out was adopted to compensate for light and temperature variations within the glasshouse.

Five replicates of each treatment were included involving first harvest year plants, and over the 2 years a completely randomized distribution of the plants within the glasshouse was employed. The same glasshouse was used for both halves of the experiment, but as the results are not fully comparable they are treated separately.

Results

The results are divided into two parts, the first dealing with seedlings and the second with mature or first harvest year plants.

Seedling plants

Stem production was markedly affected by GA, the number produced increasing with late sprayings. Single-stem types were obtained from plants treated at the 3 L and 7 L stages (Pl. 14), whilst those sprayed at the 9 L stage produced a mean of 3·8 stems per plant. The application of GA at the 3 T and 4 T stages resulted in

mean stem numbers of 5·0 and 6·4, respectively, compared with the mean of 6·9 stems derived from the controls. The above differences were all significant at the 5% level of *P*.

Branching on the lower stem nodes was promoted by all treatments, especially where GA was applied before the emergence of the 9th trifoliate leaf. The secondary branching pattern for each treatment is given in Table 1.

TABLE 1. *Number of internodes per secondary branch related to GA treatment*

	Main stem node								
Treatments	1	2	3	4	5	6	7	8	9
Control	–	–	–	3	2	1	–	–	–
3L	4	5	5	4	3	2	1	1	1
7L	5	5	4	4	3	2	1	1	–
9L	–	–	4	3	2	2	1	–	–
3T	–	–	4	3	2	2	2	–	1
4T	–	1	5	4	3	3	2	–	–

In the 3L and 7L plants (those with only a single stem) secondary branching was stimulated on the lower nodes, particularly on the first and the second. All the secondary branches produced by seedling plants were flower-bearing.

Observations on tertiary branching were made on each plant 3 weeks after emergence of the first head, and the degree of branching was found to be extremely variable. It is interesting to note that it was absent in control plants, but present in all treated plants, being most frequent in those sprayed at the 3T and 4T stages.

The data relating to emergence and head production are summarized in Table 2.

TABLE 2. *Mean emergence date and mean number of inflorescence heads per plant*

Treatments	Mean emergence	Mean heads per plant
Control	15/7	18·7
3L	13/7	12·3
7L	13/7	8·3*
9L	11/7*	10·0*
3T	10/7**	39·0**
4T	7/7**	36·0**

Significance cf. control: * $P = 0{\cdot}05$, ** $P = 0{\cdot}01$.

The advancement of flower emergence due to GA in red clover is brought about by the more rapid extension of stem internodes. Internode extension does not normally commence until seedlings have passed the sixth leaf stage, which provides an explanation for the failure of GA to advance emergence significantly in the 3L and 7L treatments.

The number of heads per plant as measured 3 weeks after the first head emerged in each group was greatly increased by the late sprayings, which were applied after tillering had commenced, and here the stimulation of head production appeared to be mainly due to the modification of branching by GA. It should be borne in

mind that the figures quoted are not a measure of the absolute heading capacity of the plant, for it is obvious that the earlier flowering induced by GA will give an enhanced value for treated plants compared with control plants, where internode extension is much slower. Such data do provide an estimate of the number of inflorescences with the maximum chance of producing seed. Earliness of flowering is the principal consideration when studying seedling crops which normally come into bloom too late in the season for adequate pollination.

First harvest year plants

Gibberellic acid applied at any time prior to internode elongation causes the plant to assume an upright habit by means of an upward curvature at the base of each tiller, a movement resembling a positive phototropic response. Late-flowering clovers normally maintain a prostrate habit until internode elongation commences.

It has also been noted that treatment at or around the critical daylength results in a cessation of tillering, similar to that demonstrated in seedling material. The number of stems produced by a plant generally bears a relationship to the number

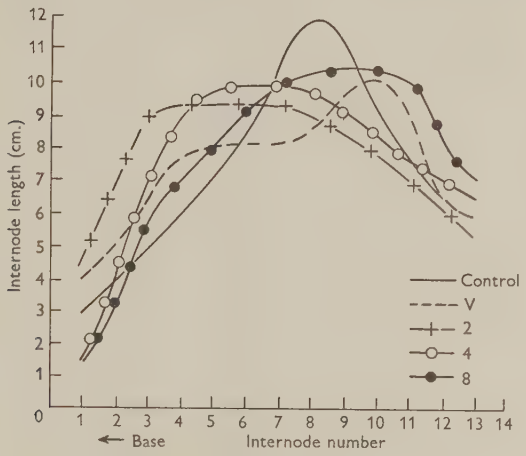
TABLE 3. *Stems per plant* (*A*), *secondary branches per stem* (*B*) *and tertiary branches per stem* (*C*)

Treatments ...	A	B	C
	1958		
Control	40·33	5·50	3·43
V	27·51**	5·37	3·03
2	33·00*	5·88	3·53
4	33·75	6·00	4·32*
8	42·10	5·94	3·93
	1959		
Control	31·50	6·65	5·70
V+2	35·75	6·75	6·50
V+4	31·25	7·00	7·15
V+8	29·25	6·30	5·25
V+10	30·00	6·20	5·25
2+4	24·50	6·00	4·05**
2+8	32·00	5·10**	4·60
2+10	28·50	6·00	5·60
4+8	23·75**	5·35*	4·80
4+10	25·50**	6·05	4·15*
V+2+4	27·25	6·70	6·65
V+2+8	32·25	7·10	7·65*
V+2+10	31·75	7·05	9·30***
V+4+8	29·00	7·45	6·00
V+4+10	35·00	6·70	6·05
V+8+10	25·25	6·10	5·65
2+4+8	31·75	5·10	4·70
2+4+10	29·00	5·65	5·70
2+8+10	29·00	4·90**	4·15*
4+8+10	27·50	5·15**	4·45*

Significance cf. control: * $P = 0{\cdot}05$, ** $P = 0{\cdot}01$, *** $P = 0{\cdot}001$.

of leaf buds forming the basal rosette at the advent of critical daylength; the timing, therefore, of the first spraying of mature plants must be very carefully controlled to avoid a reduction of stem number.

A single spraying of GA during the early stages of stem elongation depressed stem production without significantly affecting branching. Depression of mean stem numbers was significant in only two of the multiple treatment groups, although many of them showed a trend towards reduction. A concentration of treatments



Text-fig. 1. Internode extension curves.

during the middle and late stages of stem extension tended to depress the number of secondary branches per stem; this in turn produced similar trends in the frequency of tertiary branching. There was no significant increase in secondary branching with any treatment, but the values for the V+2+8 and V+4+8 groups closely approached significance at the 5% level of *P*.

Tertiary branching was significantly increased at the V+2+8 and V+2+10 treatments, especially in the latter, but the overall impression is that branching is only slightly affected by GA treatment.

'Vegetative phase' treatments increased the number of elongated internodes produced per stem, by stimulating into extension the normally short internodes found at the base of each stem. Sprayings applied after the second internode has been produced are ineffective in this respect, but they do influence the final length of internodes formed subsequently. In Text-fig. 1 effects of the timing of single GA applications on the pattern of internode extension are demonstrated.

The position on the stem of the longest internode depended upon the timing of the GA application and, with the exception of the vegetative phase treatment, was the one extended immediately after treatment. GA also affected some of the

internodes elongated prior to spraying, which showed that their normal extension fell short of the maximum possible.

An open-crown habit was developed after vegetative phase sprayings, due principally to the extension of some of the normally short basal internodes, but the earlier cessation of tillering was a contributory factor. This reduction of total leafage would undoubtedly facilitate seed harvesting.

The first inflorescence emerged earlier in all groups; the variations due to treatment are summarized in Table 4.

TABLE 4. *Mean emergence date of first inflorescence*

Treatments	Mean emergence date (June)	Days earlier than control
	1958	
Control	30·00	—
V	26·75	3·25**
2	25·50	4·50**
4	25·25	4·75**
8	26·75	3·25**
	1959	
Control	28·25	—
V+2	21·25	7·00***
V+4	21·00	7·25**
V+8	23·00	5·25***
V+10	24·50	3·75*
2+4	22·25	6·00**
2+8	21·25	7·00**
2+10	22·25	6·00**
4+8	22·00	6·25*
4+10	22·50	5·75**
V+2+4	21·00	7·25**
V+2+8	20·75	7·50***
V+2+10	20·75	7·50***
V+4+8	20·75	7·50***
V+4+10	21·00	7·25***
V+8+10	22·50	5·75**
2+4+8	21·75	6·50*
2+4+10	24·25	4·00*
2+8+10	22·50	5·75*
4+8+10	24·25	4·00*

Significance denoted as in Table 3.

Late sprayings had less effect in inducing earliness than those given before the fourth extended internode had been produced, and the maximum response was obtained with two or three sprayings in close succession during the initial stages of elongation. This is a direct reflexion of the differential rates of extension of stem internodes, for normally the later formed internodes are extended more quickly than those produced when stem elongation commences.

GA applied during the production of the first four internodes had a greater effect than applications at later dates. This evidence supports the scheme of Brian (1958),

relating to the physiological function of endogenous gibberellins in so far as it demonstrates that applied GA becomes less effective in influencing internode elongation during the later stages of growth.

The number of heads per stem was also affected by GA treatment and this was related to the head-bearing capacity of branches under different treatment regimes.

TABLE 5. *Total heads per stem at harvest (A) related to heads per stem borne on secondary (B) and tertiary branches (C)*

Treatments ...	A	B†	C
	1958		
Control	9·53	5·79	2·74
V	12·80**	8·90**	3·90*
2	11·07*	8·44*	2·63
4	8·64	6·31	2·33
8	7·60*	5·05	2·55
	1959		
Control	9·30	6·45	2·85
V+2	11·70*	8·40*	3·30*
V+4	10·30	8·00	2·30
V+8	12·70**	9·50**	3·20*
V+10	9·45	7·85	1·60
2+4	10·50*	8·50**	2·00
2+8	9·50	8·00	1·50*
2+10	10·00	7·95	2·05
4+8	8·70	6·00	2·70
4+10	10·75*	8·45**	2·30
V+2+4	11·25*	9·10***	2·15
V+2+8	13·05**	8·70**	4·35**
V+2+10	14·00***	10·05***	3·95*
V+4+8	12·60*	10·05***	2·55
V+4+10	10·80*	9·40**	1·40**
V+8+10	12·95**	9·30*	3·65*
2+4+8	9·55	7·00	2·55
2+4+10	10·40	7·30	3·10
2+8+10	10·05	7·75	2·30
4+8+10	8·65	6·95	1·70*

Significance denoted as in Table 3.
† Figures in column B include terminal inflorescences.

Significant increases in head production again appeared to be associated with early growth-stage treatments, the greatest response being in the triple spraying groups. It may also be noted that the majority of the stimulatory multiple treatments included V+2 in the spraying pattern, whilst the maximum response obtained in the single application groups followed 'vegetative phase' and second internode spraying.

These increases were due to a greater number of heads being borne on secondary or tertiary branches, or on both. It will be noticed immediately that the values in column B, Table 5, are larger than their counterparts in Table 3, although the data were collected from the same stem samples. This is due partly to the inclusion of the terminal inflorescence, but mainly to the high incidence of multiple inflorescences

in the GA-treated groups. These latter were either separate, in which case two or more heads were present in the same leaf axil, or partially fused, giving the appearance of a very large bloom. The incidence of multiple heading is tabulated below and for the purpose of clarity only non-fused multiples were scored.

TABLE 6. *Number of multiple inflorescences per stem* (1959 *data*)

Treatments	Multiple heads	Treatments	Multiple heads
Control	0·35	V+2+4	1·15**
V+2	1·85***	V+2+8	2·30***
V+4	1·50**	V+2+10	1·15*
V+8	0·50	V+4+8	1·55**
V+10	0·55	V+4+10	1·05**
2+4	1·55**	V+8+10	1·05*
2+8	1·40**	2+4+8	1·55***
2+10	1·15*	2+4+10	1·10*
4+8	1·80**	2+8+10	1·50**
4+10	1·15**	4+8+10	2·05***

Significance denoted as in Table 3.

It appears that the large increases in number of heads per stem resulted from a high incidence of double heads on secondary branches, in some instances coupled with accelerated inception of tertiary branching. The latter effect was possibly a consequence of more rapid internode extension giving an earlier decline of apical dominance in the secondary branches.

The differences in heading when discussed on a per stem basis seem small, but if we obtain the calculated heads per plant for control and V + 2 + 10 by multiplying the mean number of stems per plant by the mean number of heads per stem, the difference becomes considerable, i.e. control 292·95 heads per plant compared with V + 2 + 10, giving 444·50 heads per plant, a difference of 151·55 heads.

The use of a single clone in these studies precluded the possibility of obtaining data on the comparative seed yields of the treatment groups.

DISCUSSION

In both seedling and first harvest year plants tillering ceased shortly after GA was applied, consequently, fewer stems were produced by the treated plants. This effect reached its extreme in the single-stem types which developed if GA was given before the seventh trifoliate leaf stage.

Seedlings of red clover normally commence growth under conditions favouring floral initiation but they do not respond to the inductive daylength until they have passed the sixth-leaf stage, although they commence tillering before the emergence of the fourth leaf. Tillering continues until internode elongation begins shortly after the emergence of the seventh leaf, by which time there is a tiller in the axil of each leaf. Each tiller produces a stem, and seedling plants normally produce seven or eight stems when they bloom in the sowing year.

Arguing from the observed effects of GA upon stem production, it would appear that GA inhibits sprouting of lateral buds and brings about a premature onset of the apical dominance conditions which normally follow floral induction. A similar pattern of events was observed in mature plants where GA applied before the advent of critical daylength stopped tillering and promoted extension of the first stem internode. These effects are in accord with the hypothesis of Brian & Hemming (1958), to explain the means by which GA influenced internode extension in intact pea plants. They suggested that GA increased the effective level of indole-acetic acid (IAA) by neutralizing an 'inhibitory principle' which normally kept the response to IAA below maximum. This would also account for the sudden apparent increase in apical dominance observed in red clover following GA treatment.

Acceleration of internode elongation, leading to earlier flower emergence and earlier breakdown of apical dominance in the elongated stem, could provide an explanation of the greater development of the branch system in some of the treatment groups.

Chailakhian (1959) attributes flowering responses to a two-component system consisting of gibberellins, necessary for stem formation, and anthesins, necessary to induce the apex to change from the vegetative to the reproductive condition. He points out that stem formation is a pre-requisite for flowering in long-day species, and suggests that these fail to flower under short-day conditions because of a lack of gibberellins, whilst short-day species remain vegetative in long days due to lack of anthesins. The effects of GA upon red clover suggest that it may be a substitute for, or affect, endogenous factors controlling stem elongation, and the results quoted support the suggestion that these factors are naturally-occurring gibberellins.

Although most of the effects of GA described in this paper can be explained in terms of increased cell extension, the greater incidence of multiple heading, especially of the partially fused type, suggests that some modification of cell division may take place. This could be an indirect response resulting from imbalance in the pattern of growth regulation during flower formation, which, in turn, could be due to synergism of GA and IAA, or neutralization of inhibitory systems active in the morphogenesis of the inflorescence.

In dealing with the practical aspects of the results quoted, the responses of seedlings and first harvest year plants to GA are best considered separately.

Seedling plants resulting from an early spring sowing normally bloom in late September and owing to the infrequency of wild bees at this time very little pollination takes place. To ensure adequate pollination it would be necessary to induce heading from 3 weeks to 1 month earlier than normal, a period greatly in excess of the maximum advancement of 8 days achieved with the seedling treatments studied. Early growth stage treatments had little effect on head emergence but modifications of growth habit were induced, which would reduce the seed-yielding potential in the *following* year. It is concluded that the use of GA does not make it possible to obtain a significant seed crop from late-flowering red clover in the sowing year.

Increased earliness in first harvest year plants is not of value in itself but the shorter flowering period which results assists even ripening, whilst the greater number of heads per stem following certain treatments is an indication that with adequate pollination and successful seed setting increases in seed yield per acre may well be possible on a field scale.

Stems taken at random from broadcast seed crops have indicated that competition for light restricts branching to the upper nodes in which circumstances GA could not be expected to have a very marked effect on seed yield. On the other hand, clover crops sown in wide drills have a much more extensive branch system, and it is probable that GA would be effective in increasing seed yield.

Further work is required on the effects of competition in relation to GA treatment and field trials on this aspect are, at present, in progress at Aberystwyth.

The writer wishes to thank Professor P. T. Thomas, Director of the Welsh Plant Breeding Station, Aberystwyth, for the provision of facilities to carry out this work. Acknowledgement is also made to Mr H. M. Roberts for valuable advice on the seed production aspects of the work and to Mr J. H. Richards for the photographs reproduced in the plate.

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EXPLANATION OF PLATE 14

A. Untreated seedling plant with first flowering heads emerging. There are five stems; secondary branching has begun.

B. Single-stemmed type produced after GA treatment at the third trifoliate leaf stage. Terminal inflorescences are emerging; secondary branching is well advanced.

(*Received* 12 *March* 1960)

A

B

STODDART—*Response of red clover to gibberellic acid*

(*Facing p.* 810)

Modlibowska, Irena (1960). *Ann. appl. Biol.* **48** (4), 811–816.

BREAKING THE REST PERIOD IN BLACK CURRANTS WITH GIBBERELLIC ACID AND LOW TEMPERATURE

By IRENA MODLIBOWSKA

East Malling Research Station

(With Plate 15)

A single application of gibberellic acid (GA) was successful in breaking the rest period of black currants in September, October and November, while chilling for 2 weeks was insufficient to be effective.

The highest percentage of bud burst was obtained by GA application immediately after defoliation. Delay in GA application after defoliation decreased and delayed plant response because penetration of GA was hindered. Multiple and very early GA applications immediately after taking the plants into the glasshouse caused shedding of undeveloped buds.

In order to have black currant bushes flowering in autumn, for use in frost investigations, a study was made on methods of breaking the rest period. The effects of chilling, responsible for breaking the rest period under natural conditions (Chandler, 1925; Sergeev, Bajkov & Sergeeva, 1957), and gibberellic acid, known to substitute for chilling effects in some other plants (Chouard, 1957; Donoho & Walker, 1957; Cooper & Peynado, 1958), were tested.

Two- and three-year-old bushes of the variety Wellington XXX grown in pots were treated in September, October and November. The chilling consisted of exposure of plants for 2 weeks to a constant temperature of 2° C. in darkness. Gibberellic acid, at 50 and 100 p.p.m. with addition of Manoxol as wetter was applied with a brush to individual buds and shoots in a glasshouse. Forcing of all plants was done in a glasshouse heated to 15° C. and with supplementary light from a 500 W. combined tungsten and mercury vapour lamp to give a 16 hr. photoperiod. Experiments carried out in two seasons, 1958 and 1959 gave confirmatory results. For brevity only, the 1959 results are presented in full.

A preliminary experiment with GA showed that, although there was some interaction between individual buds on a shoot, individual shoots behaved as separate units. Therefore branches were used as basic units for GA treatments, which were usually applied to half the number of main branches on each of the three bushes in each treatment. Buds in axils of leaves that were shed naturally (commonly those at the base of each shoot) behave differently from those in axils of leaves removed immediately before treatment, and therefore their data are presented separately.

Experiment A. Forcing of twelve plants in September 1959, when 30% of their foliage was shed.

The treatments were as follows.

(1) Three bushes chilled 1–15 September brought into the glasshouse on 15 September.

(2) Three bushes brought into the glasshouse 1 September and half the branches treated with GA, 100 p.p.m. 2 weeks later, i.e. 15 September.

(3) Control (*a*), consisting of the untreated branches of the bushes listed under (2).

(4) Three bushes brought into the glasshouse 15 September and half the branches treated with GA 100 p.p.m. the same day.

(5) Control (*b*), consisting of the untreated branches of the bushes listed under (4).

(6) Control (*c*), three bushes brought into the glasshouse 15 September.

The bushes were defoliated and treated on 15 September and forced in the same glasshouse thereafter. The 2 weeks chilling was not sufficient to break the rest period, and only a few chilled buds burst. Five days after GA treatment bursting

TABLE 1. *Effect of chilling and GA on bud-burst of black currants, recorded 30 September 1959, 2 weeks after treatment*

Treatment	Buds whose subtending leaves: (*a*) Were shed naturally — Nos. buds	(*a*) Were shed naturally — % burst	(*b*) Were removed at treatment — Nos. buds	(*b*) Were removed at treatment — % burst
(1) Chilled	160	0·0	498	3·5
(2) GA 100 p.p.m. after 2 weeks in glasshouse*	125	25·1	218	86·6
(3) Control after 2 weeks in glasshouse*	93	0·0	237	7·1
(4) GA 100 p.p.m.*	98	20·4	237	89·6
(5) Control*	94	0·0	220	1·2
(6) Control	230	0·0	384	0·6

* Half the branches of each three bushes treated and half control.

TABLE 2. *Effect of GA upon floral malformation expressed in percentage of flowering trusses, 4 weeks after treatment*

Treatment	Buds whose subtending leaves: (*a*) Were shed naturally	(*b*) Were removed at treatment
(1) Chilled	0·0	0·0
(2) GA 100 p.p.m. after 2 weeks in glasshouse*	18·9	40·6
(3) Control after 2 weeks in glasshouse*	n	0·0
(4) GA 100 p.p.m.*	61·0	87·1
(5) Control*	n	0·0
(6) Control	n	n

* Half the branches of each bush treated.
n No buds showing.

of terminal buds was visible, and 14 days after treatment the differences were striking, both between GA-treated and untreated shoots and between buds naturally and artificially defoliated (Table 1). The bursting of buds treated with GA at defoliation was spectacular (Pl. 15, fig. 1), while that of earlier defoliated buds was considerably lower (Table 1 (2) and (4)). At that stage there was not

much difference between the GA treatments. Two weeks later, however, when the blossom developed, great differences appeared (Table 2). The proportion of malformed buds was much higher on bushes treated with GA immediately on being taken into the greenhouse than on those treated with GA after 2 weeks in the greenhouse. On chilled bushes, on control branches and on the whole control bushes no malformed trusses developed. On plants treated with GA immediately after being taken into the glasshouse (treatment 4) and particularly in buds whose subtending leaf was removed just before GA application, a very high proportion of malformed trusses developed. These trusses were characterized by a very strong development of the lower part of the peduncle, while the floral buds were arrested at an early stage of development (Pl. 15, fig. 2), and abscissed. Their ovaries and anthers

TABLE 3. *Effect of chilling and GA on bud-burst of black currants, recorded 30 October 1959, 2 weeks after treatment*

	Buds whose subtending leaves			
	(*a*) Were shed naturally		(*b*) Were removed at treatment	
Treatment	Nos. buds	% burst	Nos. buds	% burst
(7) Chilled	283	0·0	327	0·0
(8) GA 50 p.p.m. } after 1 week in	106	6·0	99	88·9
(9) GA 100 p.p.m. } glasshouse*	161	34·7	116	87·9
(10) GA 50 p.p.m.	103	23·6	225	79·6
(11) GA 100 p.p.m.*	122	29·7	244	84·9
(12) Control	244	0·0	423	0·0

* Half the branches of each bush used for treatment.

appeared 'hollow', indicating that ovules and pollen were affected first. This phenomenon was also observed when GA was applied several times to the same bud early in autumn. Two factors may be largely responsible for this:

(1) The high rate and amount of GA absorption through fresh leaf scars,

(2) The stage of bud development at the time of GA application. In the middle of September the buds seemed to be more susceptible to GA toxicity than later in October or November.

Experiment B. Forcing of twelve black currant bushes in October 1959 with 60% of their foliage still on.

Treatments were as follows.

(7) Three bushes chilled 1–15 October brought into the glasshouse 15 October.

(8) Three bushes brought into the glasshouse 7 October and half of the branches treated 1 week later (15 October) with GA 50 p.p.m. and (9) the remaining branches with GA 100 p.p.m.

(10) Three bushes brought into the glasshouse 15 October and half the branches treated the same day with GA 50 p.p.m. and (11) the remaining branches with GA 100 p.p.m.

(12) Three bushes brought into the glasshouse 15 October.

The bushes were defoliated and the GA treatments were applied on 15 October, after which all were forced in the glasshouse. In this experiment no buds burst on the chilled or control bushes after 2 weeks of forcing (Table 3). The GA treatment, regardless of concentration, again had a spectacular effect on the bursting of buds whose subtending leaves were removed immediately before the treatment (Pl. 15, fig. 3). On such buds, the week in the glasshouse before GA application showed no marked effect. Buds whose subtending leaves shed naturally tended to burst more readily after GA at 100 p.p.m. than at 50 p.p.m. No malformed trusses developed.

TABLE 4. *Effect of shoot tipping and GA upon bud-burst of black currants 3 weeks after treatment* 1958

Half the branches of each bush used for treatment.

Treatment		Bud burst (%)
(13) Tipped	GA 100 p.p.m.	61·2
(14) Not tipped	GA 100 p.p.m.	59·6
(15) Tipped	No GA	36·5
(16) Not tipped	No GA	18·8

TABLE 5. *Percentage bud-burst 2 weeks after GA application*

Half the branches on each bush treated.

First treatment	Second treatment	Weeks between defoliation and second treatment	Buds whose subtending leaves: (*a*) Were shed naturally	Buds whose subtending leaves: (*b*) Were removed at treatment
(5) Control	(17) GA 30 Sept.	2	13·0	15·1
(4) GA 15 Sept.	(18) No GA	0	20·4	89·6
(6) Control	(19) GA 30 Sept.	2	13·3	36·4
	(20) No GA	—	3·3	15·7
(1) Chilled	(21) GA 30 Sept.	2	11·8	35·2
	(22) No GA	—	8·1	24·9
(7) Chilled	(23) Not tipped, GA 6 Nov.	3	3·2	5·7
	(24) Tipped, GA 6 Nov.	3	6·5	20·0
(12) Control	(25) Not tipped, No GA	7½	4·0	13·5
	(26) Tipped, No GA	7½	5·3	23·2

Experiment C. On 11 November 1958 three black currant bushes, which had already shed their foliage, were taken into the glasshouse. Shoots on half of their branches had their tips cut back by about 2 cm. After 10 days of forcing there was no sign of bud burst. Then GA at 100 p.p.m. was applied to buds on half the branches of the bushes, including both tipped and untipped shoots. The record taken 3 weeks later (Table 4) shows that GA application considerably increased bud-burst over the controls. Tipping of shoots greatly increased the proportion of bud-burst on the control shoots but did not affect it on GA treated ones.

Experiment D. As neither chilled (treatments 1 and 7) nor control (treatments 5, 6 and 12) plants and shoots in experiments A and B had broken their rest period

satisfactorily, GA at 100 p.p.m. was applied to their buds 2 and 3 weeks after the plants had been taken into the glasshouse and defoliated. The bud-burst record taken 2 weeks after GA treatment (Table 5) showed a considerable reduction of its effectiveness in comparison with previous experiments (Tables 1, 3).

Treatments (17) and (18) in Table 5, applied to different branches of the same three bushes, show a striking difference in bud burst (Pl. 15, fig. 4). In this case, however, there might have been some inhibiting effect of the developing half of the bush upon the dormant half treated later. In all other treatments the low percentage of bud-burst was shown (Table 6) to be due rather to the delay in response to GA than to lack of it. The greater response to GA when applied to fresh leaf scars, than when applied to the healed scars of naturally shed leaves suggested that penetration of the GA was a crucial factor. A subsequent test was therefore made with GA injected with a hypodermic syringe into 136 buds and painted on 124 buds. Water was painted on 117 buds, but no water-injected buds were included. Within 14 days 46 % of the GA-injected buds had burst, only 11 % of the GA painted ones and 6 % of the water painted ones, again suggesting that increased entry of GA gave a greater effect.

TABLE 6. *Percentage bud-burst 3 weeks after GA application*

Half the branches on each bush treated.

Treatment	Weeks between defoliation and the treatment	Buds whose subtending leaves (*a*) Were shed naturally	Buds whose subtending leaves (*b*) Were removed at treatment
(19) GA 30 Sept.	2	36·2	50·3
(20) No GA	—	8·1	18·3
(21) GA 30 Sept.	2	31·4	51·7
(22) No GA	—	11·1	29·4
(23) Not tipped, GA 6 Nov.	3	58·4	67·8
(24) Tipped, GA 6 Nov.	3	72·3	83·4
(25) Not tipped, No GA	7½	4·0	15·3
(26) Tipped, No GA	7½	10·2	33·0

DISCUSSION

Plants in their 'rest' period, in contrast to those 'dormant', can be stimulated into growth only after breaking of the rest mechanism. In the present experiments GA proved a powerful and rapid breaker of the rest period in black currants at a time when chilling cannot be applied for a long enough period to be effective.

GA was most effective at the beginning of the rest period and less so towards its middle. The effectiveness of GA depended largely upon its penetration into the bud. Buds treated immediately after defoliation or those injected with GA responded most readily. Too high GA concentration within a black currant bud in its early stage of development caused withering and abscission of the floral buds.

Terminal buds as a rule broke their rest period most readily and then inhibited the development of the lower buds (Pl. 15, fig. 4). Their removal increased bud breaking. Early GA application also broke the apical dominance.

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EXPLANATION OF PLATE 15

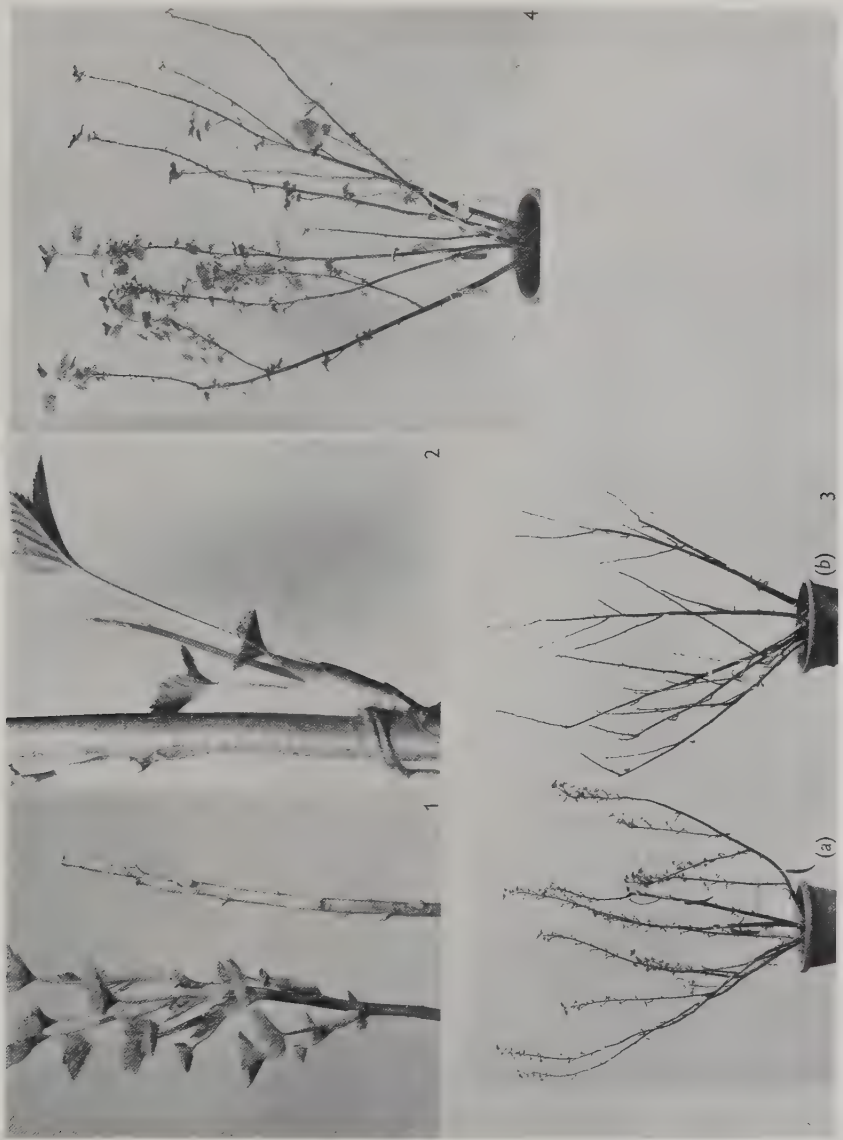
Fig. 1. Bud-burst on black currant shoots on 30 September 1959. Left, 2 weeks after GA application. Right, lack of bud-burst following 2 weeks of chilling and 2 weeks in glasshouse.

Fig. 2. An abnormal inflorescence on a black currant caused by GA toxicity.

Fig. 3. Black currant bushes on 6 November 1959, (*a*) 3 weeks after GA application, (*b*) untreated. The right half of bush (*a*) received 100 p.p.m. GA, and the left half, 50 p.p.m.

Fig. 4. Bud burst one month after forcing. The left half of the bush received GA at defoliation, the right half 2 weeks after defoliation. Note development on the right confined to terminal buds.

(*Received 22 March* 1960)



MODLIBOWSKA —*Breaking the rest period in black currants*

(*Facing p.* 816)

Ebben, M. H. (1960). *Ann. appl. Biol.* **48** (4), 817–823.

BROWN ROOT ROT OF TOMATOES

III. THE BACTERIAL FLORA OF THE RHIZOSPHERE

By M. H. EBBEN

Glasshouse Crops Research Institute, Rustington, Sussex

(With 2 Text-figures)

Population counts and nutritional grouping of bacteria from a tomato-sick soil showed that steaming greatly reduced the population and the percentage of group I bacteria (i.e. those able to use inorganic nitrogen). The numbers subsequently increased, and by the time tomatoes were planted, the distribution of nutritional groups approximated to that in unsteamed soil. Rhizosphere and root-surface population numbers did not differ widely in steamed and unsteamed soils. The nutritional groups in soil before planting differed much more between samples from steamed soil with a changing population, than from soil unsteamed for 3 years. Groups I and II (bacteria requiring amino acids) were higher in unsteamed, cropped soil than in steamed soil, and were dominant in rhizosphere and root-surface populations. Group I increased on the root surface in unsteamed soil from July to October, whereas group II increased on root surfaces in steamed soil. Group V (bacteria requiring soil extract) was less frequent on the root surface than in soil from plots unsteamed for 1 year, but more frequent on the root surface than in soil unsteamed for 3 years.

Early work on the bacterial flora of soils and rhizospheres dealt with population numbers and identified the bacteria as far as possible by standard methods. A different system, classifying soil bacteria into nutritional groups, was suggested by West & Lochhead (1940*a*) and Lochhead & Chase (1943). This entails isolating bacteria from soil on a non-selective medium, subculturing each isolate on a range of media of increasing nutritional complexity, and grouping together the bacteria with the same requirements for good growth. West & Lochhead (1940*a*) further suggested that a Bacterial Balance Index (BBI) of a particular habitat could be evaluated by giving the percentage of bacteria capable of growth in the simplest medium a negative value, and the percentage requiring more complex nutrients a positive value. Differences in the BBI between different habitats or changes within one habitat would give a better idea of the bacterial activity than population figures alone.

The nutritional grouping of soil bacteria has been extensively used and further developed, especially by Canadian workers, in connexion with strawberry, tobacco and flax root diseases (Hildebrand & West, 1941, West & Lochhead, 1940*b*; Katznelson & Chase, 1944; Katznelson & Richardson, 1948). West & Lochhead (1940*a*) stated that strawberry root-rot soils have a lower BBI than healthy soils, and that the BBI of rhizosphere soil is higher than that of control soil. Hildebrand & West (1941) found that rhizosphere populations from diseased strawberry roots had a lower BBI than those from healthy roots. Lochhead (1940) suggested that resis-

tance to soil-borne diseases is linked with selective action of root excretions upon saprophytes in the rhizosphere, favouring those types antagonistic towards pathogenic organisms. Further work has shown that the BBI itself is less useful in comparing two populations than the changes in the proportions of the nutritional groups, because the actual figure may mask simultaneous changes in several groups. Taylor (1951) suggested modifying the media used and reducing the number of nutritional groups, and his suggestions have been adopted in the investigation described below.

This work on the bacterial flora of tomato rhizospheres was done jointly with that on brown root rot of tomatoes in a glasshouse soil, reported by Ebben & Williams (1956) and Ebben (1959).

Methods

Bacterial counts and isolations from soil and rhizospheres were made from plots in a commercial glasshouse where tomatoes had been grown for 30 years, the soil steamed at least every 2 years. The variety E.S. 5 was grown under normal commercial conditions in five plots. Treatment of the plots before planting was as follows; plots A and B had been left unsteamed for 1 year, C and D were comparable plots but were steamed by Hoddesdon pipes 4 months before planting in March, and plot E had been unsteamed and cropped for 3 years. In the previous year plots A and C had been fallowed, and B and D cropped. Bacterial counts were made from composite soil samples at a final dilution of 1 in 10^6, on eight plates of a soil-extract-yeast-tryptone agar (Taylor, 1951). Outer rhizosphere soil samples were obtained by shaking soil from around the roots of three plants, mixing and treating as for a soil sample. Root-surface population counts were made by shaking brushed root segments in sterile water, diluting and plating the suspension (Ebben, 1959). After 5 days incubation at 25° C. colonies were counted and all the colonies on one typical plate of a series (about fifty) were subcultured into yeast-tryptone semi-solid agar (0·3%). Each isolate was transferred to two tubes of each of five different broths for determining their nutritional groups (Taylor, 1951). The cultures were incubated for 10 days at 25° C., and growth was then graded by eye 0–4, according to the turbidity. Each isolate was then assigned to the group corresponding to the simplest medium in which there was maximum growth. The grouping was as follows:

Group I, bacteria able to use inorganic nitrogen.

Group II, bacteria requiring amino acids.

Group III, bacteria requiring amino acids and growth factors.

Group IV, bacteria requiring tryptone.

Group V, bacteria requiring soil extract.

Soil samples for nutritional grouping of bacteria were taken in January, February and March, before planting, and again in May before the soil was permeated with plant roots. Population counts only were made from an April sampling. Rhizosphere samples and soil samples from between the rows of plants were taken in July and October.

Results

Table 1 shows the numbers of soil bacteria calculated from dilution plates in samples taken early in the season.

Steaming drastically decreased the bacterial population, but there was a rise in numbers after soil flooding in February in all plots, except possibly E, and in the steamed plots this increase was very large, bringing the bacterial counts up to the level in the unsteamed plots. This increase in number was maintained at the next two samplings, possibly due to rising soil temperatures. Bacterial numbers in plot E for the five samplings January–May were significantly higher (0·5% level) than in plots A and B left unsteamed for only 1 year.

Table 1. *No. of bacteria in millions/g. of dry soil*

	Unsteamed 1 year		Steamed		Unsteamed 3 years
Plots ...	A	B	C	D	E
Jan.	85·7	28·3	1·0	0·7	167·3
Feb.	35·8	43·4	3·9	6·6	79·6
Mar.	105·2	84·5	103·7	204·8	114·8
Apr.	150·9	110·7	113·1	149·4	197·5
May	111·4	108·8	99·5	228·0	145·7

Note. All plots flooded in February and planted in March.

Table 2. *No. of bacteria in millions from soil, rhizosphere and root surface*

Plots ...	A	B	C	D	E
			July		
Soil	84·9	60·3	88·7	83·1	63·7 } per g. dry soil
Rhizosphere	131·9	154·9	144·6	146·8	74·5 } per g. dry soil
Root surface	193·3	305·1	137·3	254·7	169·0 per g. air-dry root
			Oct.		
Soil	46·0	50·9	64·2	52·4	26·3 } per g. dry soil
Rhizosphere	81·2	43·5	148·8	94·7	69·4 } per g. dry soil
Root surface	562·3	64·3	156·0	162·4	150·6 per g. air-dry root

Table 2 shows that when the first rhizosphere samples were taken in July, soil bacterial numbers dropped in all plots. Counts from rhizosphere soil were always higher than those from soil in July; the ratio of outer rhizosphere to soil numbers ranged from 1·16 to 2·56 in the five plots. Numbers of soil bacteria were lower in October than in July, the rhizosphere counts from most plots also decreased; the ratio of outer rhizosphere to soil numbers ranged from 0·8 to 2·6. Root surface populations were on average highest in plots A and B, and lowest in plot E.

The relative frequency of the nutritional groups as percentages of the isolates tested is shown in Figs. 1–2.

The first soil samples from the steamed plots gave very few group I bacteria, and relatively many group V; later samples showed populations with simpler nutritional requirements, and the percentage of group I in particular rose steadily

from January to May. In the unsteamed plots group V was the smallest, and groups I and II usually accounted for approximately 50% of the population. No differences were noted after flooding the soil, but after planting, at the fourth sampling, there

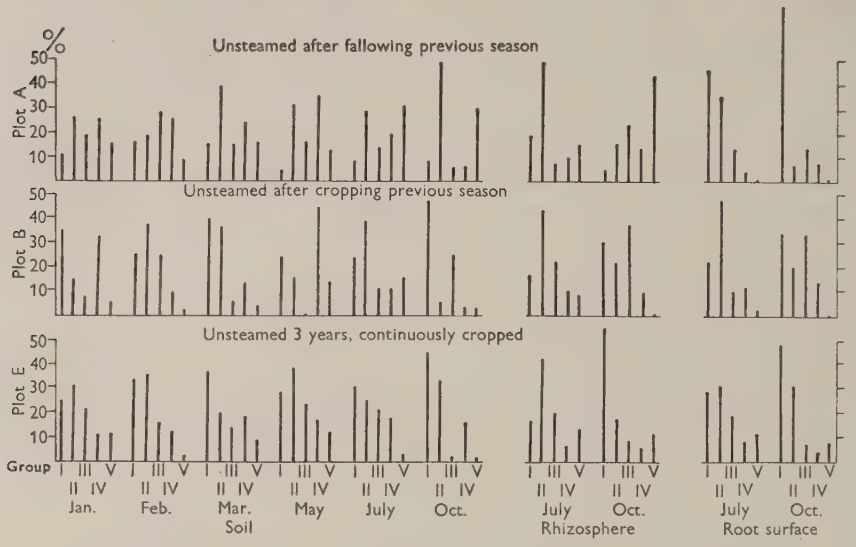


Fig. 1. Nutritional groups of bacteria associated with tomatoes.

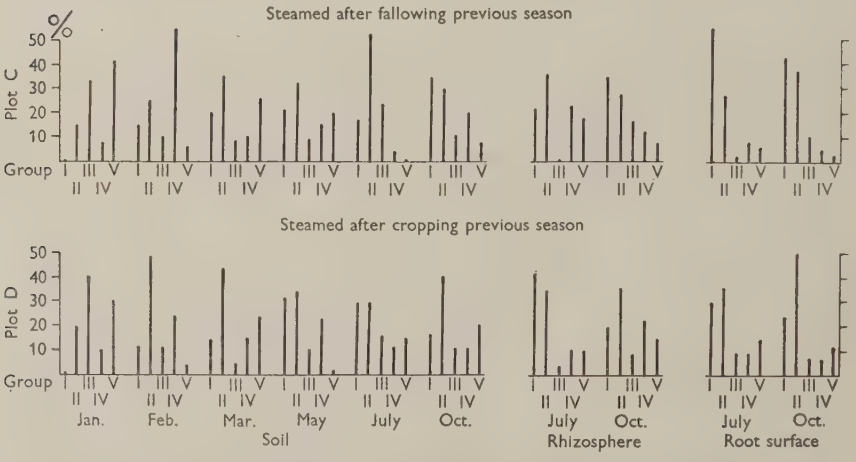


Fig. 2. Nutri ional groups of bacteria associated with tomatoes.

was an increase in group IV, but not in the steamed plots C and D, nor in plot E. The steamed plots showed a greater range between samples within individual groups, with a higher proportion of groups III–V than in plots A and B, where

groups I, II and IV were dominant, especially in plot B which had been cropped the previous year. Plot E showed less variation between samples within the individual groups than other plots and there was a decided dominance of groups I and II bacteria.

The distribution of nutritional groups in the soil, rhizosphere and root-surface samplings from the five plots in July and October shows the same general pattern, i.e. dominance of groups I and II. In all except plot D, group I increased in soil from July to October. The root-surface populations in plots A, B, and E also showed an increase in group I from July to October, but in the steamed plots group I decreased in this period although group II increased. Group III bacteria, those requiring growth factors, changed little, but tended to increase from July to October in the rhizosphere and root-surface samples from plots A to D; in plot E there was a decrease in group III from July to October. Group IV bacteria were not markedly affected by soil treatment or habitat. Group V, bacteria requiring soil extract, were more common in soil than in rhizosphere or root-surface samples in plots A and B, and slightly decreased in numbers from July to October in plots B and E. In plot E, group V was higher in the rhizosphere and root-surface populations than in the soil, whereas in plots C and D distribution of this group was not very different in different habitats. Changes in the bacterial populations from the nutritional angle did not appear to be related to previous soil treatment, i.e. steam sterilization, nor were any differences noticed between steamed and unsteamed plots in July, when root rotting in steamed plots was only slight, but in unsteamed plots was quite severe. The differences in the proportion of the nutritional groups present was more dependent on the habitat and season.

Discussion

The effect of steaming on the tomato soil studied was to decrease drastically the number of the bacteria, but re-infection and multiplication was rapid after soil flooding. The high percentage of group V present after steaming suggests that this group is stimulated by substances similar to those in artificially prepared soil extract. As the bacterial population increased, the percentage of the groups became similar to that in the unsteamed plots, i.e. with groups I and II dominant, although group V remained relatively high in the steamed plots. The increase in group I may be due to inorganic nitrogen increasing as nitrifying bacteria re-infect the soil. In the steamed plots, groups IV and V tended to vary inversely as if increase of one group created conditions unfavourable for itself but favourable to the other group. This effect did not occur in the unsteamed plots where group V was usually the smallest.

The soil population in plot E which had been left unsteamed for 3 years appeared more stable than in other plots, for there was less variation within groups at different sampling dates, whereas the steamed plots C and D showed the widest differences within groups from sample to sample. Differences in the percentage of bacterial groups in different samples of a field soil were shown by Wallace & King (1954).

It is possible that short-term fluctuations of an individual group in the soil follow the usual bacterial population growth-curve pattern, and that the results obtained from one samplir g depend on the stage of development of the population at that time. In a stable soil type, the maximum numbers of a group would be determined by soil nutrients and association effects of other bacteria, but with continued bacterial activity and modification of the habitat some change might be expected in the whole character of the population. However, Katznelson & Chase (1944) reported that the average incidence of groups over a period was similar in field soils differently manured, and they suggested that bacterial groups are not permanently or appreciably changed by soil treatment. In the work described above, there was general dominance of groups I and II in all plots, with a tendency for a decrease in groups III, IV and V from July to October. The steamed soils rapidly developed a flora similar to the other plots. Most workers have reported an increase in group II bacteria, those requiring amino acids, on plant roots compared with soil. This effect was not found in this work, even when comparing unplanted soils early in the year with root-surface populations developing later. In the unsteamed plots where root rotting was most severe group I increased on the root surface from July to October, whereas in the steamed plots group II increased. This might reflect a difference between diseased semi-moribund roots and living root surfaces. Hildebrand & West (1941) quoted a similar effect for strawberry root rot where approximately 50% of the bacteria on diseased roots were those requiring inorganic nitrogen, and Katznelson & Richardson (1943), working with young tomato plants, found an increase in the percentage of group I bacteria in the rhizosphere compared to control soil population, especially in unsterilized soil.

The difficulty in comparing results of bacterial counts and nutritional grouping of rhizosphere populations reported by different workers for different crops was mentioned by Clarke (1947) and Smith (1948). Methods of sampling root-surface populations differ in the amount of attached soil included in the 'root' sample and in whether the population is calculated per unit weight of root or associated soil.

The large 'rhizosphere effects' reported could be due to the lack of a strictly comparable method for estimating soil and rhizosphere floras. Tesić & Todorović (1958), calculating rhizosphere populations on a 'per gram of dry root matter' basis, claimed that for wheat and beans a rhizosphere effect was found in poor soil but not in fertile soil. They suggested that when the soil microbial activity is low the rhizosphere provides a more favourable environment, but in fertile soil where the population is higher this does not necessarily follow.

Different crops, age of plant, and disease susceptibility may influence the rhizosphere flora (West & Lochhead, 1940*b*; Wallace & King, 1954; Lochhead, Timonin & West, 1940). A bacterial flora which develops in the soil in response to the soil type may change conditions for other organisms by competition, synthesis of nutrients or antagonism. These effects may be exerted on any plant pathogens present, but it seems as likely that the different bacterial equilibria in different soils may be only indicators of conditions acting directly on the pathogens

(West & Hildebrand, 1941). Similarly the root exerts its own influence on the microbial population, including any pathogen present. In this study differences in the bacterial populations which could be correlated with the development of brown root rot were not evident. The rapidity with which symptoms developed, even on steamed soil, make it probable that any differences between the rhizospheres of healthy and diseased roots would only be evident at an earlier stage of plant growth.

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STUDIES ON *GLOEOSPORIUM MUSARUM* CKE. & MASSEE CAUSING STORAGE ROTS OF JAMAICAN BANANAS

III. CONTROL WITH SODIUM SALICYLANILIDE ('SHIRLAN WS') AND NYSTATIN

BY D. S. MEREDITH

Banana Board Research Department, Kingston, Jamaica, W.I.

(With Plate 16)

Gloeosporium musarum Cke. & Massee is the most important fungus associated with finger-stalk rot of Jamaican Lacatan bananas, *Fusarium* spp. being of minor importance. Sodium salicylanilide (Shirlan WS), at concentrations of 0·5, 1·0 and 1·5 %, provided approximately 30, 50 and 65 % control, respectively, when inoculated fruit was dipped for 1 min. in the compound: severe phytotoxic effects were caused by all concentrations. When Shirlan WS treatment was followed by rinsing in water, there were no phytotoxic effects and approximately 15, 35 and 40 % control of finger-stalk rot, respectively, was obtained. 1·0 and 1·5 % Shirlan WS also effected good control of anthracnose (*G. musarum*), both with and without post-treatment washing.

Moderate to poor control of *Gloeosporium* rot was obtained when Shirlan WS/washing treatment was delayed for more than 24 hr. after inoculation. A 2 min. immersion period in Shirlan WS was more effective than shorter periods: a momentary dip was ineffective.

The antibiotic nystatin (200 and 400 p.p.m.) had no significant effect on the incidence of finger-stalk rot: also, the severity of main-stalk rot was not reduced appreciably.

Practical considerations arising from the possibility of commercial-scale treatment with Shirlan WS are discussed.

INTRODUCTION

Earlier papers reported reasonable control by the antibiotic nystatin (Brown & Hazen, 1957) of banana anthracnose caused by *Gloeosporium musarum* Cke. & Massee (Meredith, 1960*a*, *b*). This fungus also causes other types of fruit-rot during transportation and storage of bananas. Further experiments carried out in an attempt to formulate methods of control are described in this paper. The Lacatan variety of banana was used in all experiments.

FINGER-STALK ROT

Nature and cause of rot

Finger-stalk rot, commonly referred to within the banana industry as 'Santa Marta stem-end rot', has been described by Wardlaw (1935). When a finger is forced towards or away from the main-stalk, or twisted, the strain imposed on the relatively slender finger-stalk may result in tissues being bruised, if not ruptured. On harvested Jamaican bananas, finger-stalk injury usually takes the form of a

transverse creasing or notching (Pl. 16, fig. 1). Infection of wounds by various fungi, in particular *G. musarum* and *Fusarium* spp. (Meredith, 1959), is followed by rotting of the stalk. At the time of discharge in the United Kingdom, finger-stalk rot may be so advanced that it is difficult to handle fruit without causing 'finger-drop' (Pl. 16, fig. 2). Further 'dropping' occurs in the ripening room where the rate of rotting increases at temperatures more favourable for fungal growth (65–75° F. as opposed to 53–56° F. inside the ship's hold). Tomkins (1931) considered 'finger-drop' to be the most important source of wastage of Brazilian fruit of the Cavendish variety: sometimes this is true for Jamaican Lacatan bananas (Elders & Fyffes Ltd., 1957).

In Australia, where bunches are divided into individual fingers ('singles') for marketing purposes, a rot known as 'black-end' has caused wastage (Simmonds, 1928). 'Black-end' is a decay of the severed stalk and the adjacent part of the finger. The disease is caused by *G. musarum* and *Fusarium* spp. (Simmonds & Mitchell, 1940) and, in this respect, is similar to finger-stalk rot of fruit that is ripened on the bunch.

Relative importance and pathogenicity of associated fungi

Observations made in the United Kingdom on finger-stalk rot showed that *G. musarum* is most frequently associated with this disease (Meredith, 1959): in a few cases, only *Fusarium* spp. are isolated. Investigations by Hoette (1935) and Simmonds & Mitchell (1940) on 'black-end' suggested that *Fusarium* spp. are secondary invaders of tissues that are already colonized by *G. musarum*. Further, these workers found that *Fusarium* spp., when inoculated into severed finger-stalks, caused slight 'black-end' and not extensive rotting as in the case of *G. musarum*. Similar observations were made on finger-stalk rot. In the present investigation, inoculations were carried out to test the relative pathogenicity of *G. musarum* and *Fusarium* spp. isolates, obtained from naturally infected fruit and it was found that whereas *G. musarum* had high rotting activity, several unidentified species of *Fusarium* caused very little decay with no tendency for 'finger-drop' to occur. Tests with mixed inocula confirmed Hoette's (1935) finding that a pure inoculum of *G. musarum* causes as much rotting as a mixed inoculum.

Inoculum sources

Leach (1958) concluded that a high proportion of finger-stalk rot observed on Jamaican bananas in the United Kingdom developed from injuries caused by mishandling during transportation from plantations to loading wharves: further damage may be caused during loading and ocean transportation. Thus infection by *G. musarum* may occur at various stages in the journey of the banana and it was desirable to obtain information about possible sources of inoculum.

Recently harvested bunches of fruit were brought direct from the field to the laboratory where they were divided into individual hands by severing the main-stalk above and below each cushion. Three lots of ten replicate hands were assorted randomly. One series was surface-sterilized by immersion for 1 min. in 70%

alcohol and allowing to dry: another series was immersed for 1 min. in tap water, while the third series was not treated. Ten finger-stalks on each hand were scratched lightly with a sterile needle. Hands were then enclosed in perforated Polythene bags and stored for 12 days in a cool-storage chamber at 54° F. After ripening for 7 days in another chamber at 68° F., the incidence of finger-stalk rot on surface-sterilized, water-treated and untreated fruit was 8, 56 and 68%, respectively, (L.S.D. at $P = 0{\cdot}001$, 42·2), suggesting that abundant inoculum of *G. musarum* was present on the fruit surface at the time of harvest. Examination of centrifuged washings from the surface of unharvested fruit have demonstrated repeatedly the presence of large numbers of viable conidia on fingers, finger-stalks and main-stalks.

In the plantations, the chief source of conidia are numerous acervuli which develop on the petiole and upper regions of the sheath of dead or dying banana leaves colonized saprophytically by *G. musarum*. Since the conidia are enveloped in slime, it is unlikely that wind currents alone can dislodge them but rain or dew droplets falling on an acervulus (Gregory, Guthrie & Bunce, 1959) would aid dispersion of conidia from petioles to inflorescences. Also, as leaves die they collapse and sometimes rest on the fruit: direct transfer of conidia in this manner has been observed. Further contamination of fruit may occur during transportation in Jamaica, since lorries and railway trucks are lined with spore-bearing 'trash' (i.e. pruned dead or dying leaves) which is also interspersed among the bunches.

The relative importance of these inoculum sources in relation to disease is not yet known. An account of more detailed studies on the dispersal of *G. musarum* will be presented elsewhere.

Experiments with Shirlan WS and nystatin

Salicylanilide (Shirlan AG) and its water-soluble sodium salt (Shirlan WS) are used commercially in Australia to control squirter disease (*Nigrospora sphaerica*) and 'black-end' (Simmonds & Mitchell, 1937, 1940; Magee, Eastwood & Foster, 1939; Magee, 1939, 1941; Anon., 1949; O'Neill, 1949; Simmonds, 1949). More recently Andrade, Puzzi & Torres (1956), investigating methods of applying Shirlan WS, concluded that spraying the complete bunch soon after harvest was the best way of controlling main-stalk rot and finger-stalk rot caused by *Ceratocystis* (*Thielaviopsis*) *paradoxa* and *G. musarum*, respectively.

Castellani, Beccari & Matta (1958) reported that nystatin reduced the amount of *Gloeosporium* rot on bananas shipped from Somaliland to Italy. The studies of Meredith (1960*a*, *b*) have been referred to already.

Experiments were set up to test the effect on finger-stalk rot of treatment with Shirlan WS and nystatin.* Aqueous solutions of Shirlan WS, at concentrations of 0·5, 1·0 or 1·5%, and suspensions of nystatin containing 200 or 400 p.p.m.

* These experiments were made with the agricultural grade of nystatin (Mycostatin 20) manufactured by E. R. Squibb and Sons, Division of Olin Mathieson Chemical Corporation, New Brunswick, New Jersey.

antibiotic were prepared: a secondary alcohol sulphate wetter was added to each dipping medium. A control was provided by water plus wetter only. Twelve sets of ten replicate hands were selected randomly and the finger-stalks were sprayed with a suspension of *G. musarum* containing approximately 2×10^3 spores/ml. After allowing the fruit to dry, finger-stalks were wounded by bending the finger towards the cushion until the peel ruptured slightly and latex flowed from the stalk: ten fingers on each hand were inoculated in this way. Hands were enclosed in air-tight polythene bags and incubated for 3 hr. at field temperature (76–80° F.). Fungicide treatment was carried out by immersing fruit for 1 min. in water or one of the nystatin suspensions, and then for a further 1 min. in water or a Shirlan WS solution. The experimental design was factorial, all possible combinations of water, nystatin and Shirlan WS being tested. The incidence of finger-stalk rot was recorded after cool storage and ripening in the manner described already (p. 826). The results are given in Table 1.

TABLE 1. *Effect of successive treatments with* (*a*) *nystatin and* (*b*) *Shirlan WS on the incidence of finger-stalk rot caused by* Gloeosporium musarum: *percentage numbers of stalks rotted*

% Shirlan WS	Nystatin (p.p.m.) 0	200	400	Mean
0	100	92	99	97·0
0·5	52	36	40	42·7
1·0	32	21	23	25·3
1·5	15	21	17	17·7
Mean	49·8	42·5	44·8	

Analysis of variance

Source	D.F.	M.S.
Shirlan WS	3	384·16***
Nystatin	2	5·51
Interaction	6	2·56

*** Significant at 0·1 % level of probability.

In assessing the results of inoculation, a plus or minus system was employed. A positive result was recorded when there was obvious finger-stalk rot, however slight, spreading from the wounded area. Rotting was usually extensive, advancing along the proximal end of the finger and also into the cushion: stalk tissues were considerably shrunken and many acervuli developed on the surface. Merely lifting a hand was often sufficient to cause 'finger-drop'. A negative result was recorded when there was a localized blackening, but no sign of decay, at the inoculation wound—a normal reaction due to polyphenol oxidase activity (Griffiths, 1959).

The results demonstrate a significant and appreciable reduction in finger-stalk rot by Shirlan WS treatment. Concentrations of 1·0 and 1·5 % gave significantly better control than 0·5 %. There was no significant effect on disease by nystatin.

However, at all concentrations tested, Shirlan WS caused moderate to severe blackening of the peel, particularly where tissues were already injured before treatment. Similar phytotoxic effects of Shirlan on Lacatan bananas were observed by Leach (1956) who concluded that the compound was unsuitable for commercial use.

In another experiment, the order of dipping in the two compounds was reversed: other details were as in the previous experiment. Results are shown in Table 2.

TABLE 2. *Effect of successive treatments with* (*a*) *Shirlan WS and* (*b*) *nystatin on the incidence of finger-stalk rot caused by* Gloeosporium musarum: *percentage numbers of stalks rotted*

% Shirlan WS	Nystatin (p.p.m.) 0	200	400	Mean
0	89	77	70	78·7
0·5	67	47	55	56·3
1·0	41	58	40	46·3
1·5	41	39	41	40·3
Mean	59·5	55·2	51·5	

Analysis of variance

Source	D.F.	M.S.
Shirlan WS	3	85·14***
Nystatin	2	6·41
Interaction	6	7·77

*** Significant at 0·1 % level of probability.

Once more, Shirlan WS significantly reduced the incidence of finger-stalk rot. Partial removal of the compound by a second dip was probably responsible for the poorer percentage control relative to that in the preceding experiment. Removal or dilution would account also for the absence of phytotoxic effects. Nystatin again caused no significant reduction in rotting.

Further information about nystatin was obtained from reports on the condition of shipments of treated bananas. Experimental details and the method of assessing disease have been given elsewhere (Meredith, 1960*b*). However, it may be noted here that, after harvest, fruit was dipped for approximately 1 min. in 200, 300 or 400 p.p.m. nystatin, to which a wetter was added. Disease indices for finger-stalk rot, observed on ripe fruit, are given in Table 3.

It is clear that nystatin had no appreciable effect on finger-stalk rot, thus confirming the results of laboratory experiments.

Two other experiments were set up to explore further the possibility of controlling finger-stalk rot with Shirlan WS, applied after different incubation periods. Experiments were designed as 4×4 factorials with ten replicate hands (each with ten inoculated fingers) for every treatment combination. The treatments were Shirlan WS, at concentrations of 0, 0·5, 1·0 and 1·5 % (plus a wetter), applied after 0, 12,

24 or 48 hr. incubation at field temperature and 98–100% R.H. In Exp. A, fruit was transferred to the cool-storage chamber immediately after 1 min. immersion in Shirlan WS or water (control). In Exp. B, fruit was washed thoroughly with tap water after Shirlan WS treatment. Results obtained after cool-storage and ripening are set out in Table 4.

TABLE 3. *Experimental shipments of bananas to test the effect of nystatin on the development of finger-stalk rot*

Date of shipment	Concentration of nystatin p.p.m.	Disease index	
		Treated	Untreated
15. ix. 59	200	24·7	27·7
23. ix. 59	300	25·7	29·3
29. ix. 59	400	27·5	32·5
5. x. 59	200	Not recorded	
15. x. 59	300	28·7	27·7
21. x. 59	400	26·7	28·5

TABLE 4. *Effect on the development of finger-stalk rot of Shirlan WS applied at intervals after inoculation: percentage numbers of stalks rotted*

% Shirlan WS	Incubation period (hr.) 0	12	24	48	Mean
	Exp. A. Shirlan WS not washed off				
0	100	100	99	100	99·7
0·5	65	54	55	72	61·5
1·0	38	42	33	48	40·3
1·5	37	47	26	35	36·3
Mean	60·0	60·8	53·3	63·8	
	Exp. B. Shirlan WS washed off				
0	100	99	98	100	99·3
0·5	89	55	86	100	82·5
1·0	64	46	55	85	62·5
1·5	51	44	54	79	57·0
Mean	76·0	61·0	73·3	91·0	

Source	Analysis of variance D.F.	M.S. Exp. A	Exp. B
Shirlan WS	3	338·02***	139·41***
Incubation	3	7·85	70·67***
Interaction	9	3·66	11·01*

* Significant at 5% level of probability: *** at 0·1% level.

In both experiments, the variance ratio for Shirlan WS treatment/error was highly significant: percentage control increased with increasing concentration of fungicide, but there was no significant difference between the results for 1·0 and 1·5% Shirlan WS. Better control was apparent on unwashed than on washed

fruit. On the former, incubation periods of up to 48 hr. had no significant effect on control but, on washed fruit, the results suggest that poor control would be expected if treatment is delayed for more than 24 hr. after inoculation. Phytotoxic effects were apparent on unwashed but not on washed fruit.

In the experiments described so far, approximately 1 min. was allowed for immersion of fruit in Shirlan WS. The effect of shortening or lengthening this period was investigated in another factorial experiment. Fruit was inoculated and then incubated for 0, 24 or 48 hr. before dipping momentarily or for 0·5, 1 or 2 min. in 1·5% Shirlan WS or water (control). Shirlan WS-treated fruit was then washed as in Exp. B above. Since there were twenty-four different treatment combinations, the number of replicate hands (each having ten inoculated fingers) was reduced to eight for convenience. The data obtained at maturity are given in Table 5.

TABLE 5. *Effect on finger-stalk rot of Shirlan WS treatment followed by a water wash: percentage numbers of stalks rotted (out of 80)*

Fungicide treatment	Incubation between inoculation and treatment (hr.)	Duration of treatment (min.) Momentary	0·5	1	2	Mean
1·5% Shirlan WS	0	58	25	35	11	32
	24	84	74	66	69	73
	48	89	75	91	71	82
	Mean	77	58	64	50	
None (control)	0	88	93	96	95	93
	24	93	96	98	95	96
	48	100	100	93	98	98
	Mean	94	96	96	96	

Analysis of variance (Shirlan WS data only)

Source	D.F.	M.S.
Incubation	2	221·07***
Immersion period	3	31·25***
Interaction	6	6·24

*** Significant at 0·1% level of probability.

The data for control fruit were not included in the statistical analysis since there was a high and more or less uniform incidence of infection. It was quite clear that the overall effect of Shirlan WS treatment was to reduce the incidence of rotting and there was a highly significant difference between the results for unincubated and incubated fruit ($P < 0{\cdot}01$). The mean square for immersion period was also highly significant, no doubt due to the tendency for percentage control to increase with increasing periods. Immersion periods of 0·5 min. or longer gave better control than a momentary dip ($P < 0{\cdot}01$).

In the experiments described so far, *Fusarium* spp. sometimes developed with *G. musarum* on rotted stalks. However, on Shirlan WS-treated fruit, no instance of finger-stalk rot caused by *Fusarium* spp. alone was observed, indicating that

Shirlan WS is no less fungitoxic to *Fusarium* spp. than to *G. musarum*. From the commercial standpoint this is an obvious advantage over nystatin, for the studies of Castellani *et al.* (1958) indicated that the antibiotic did not control *Fusarium* rot.

Anthracnose

Experiments with Shirlan WS

A series of experiments parallel to those described in the previous section were performed in an attempt to control anthracnose. From studies on the infection biology of *G. musarum*, it was suggested (Meredith, 1960*a*) that the form of anthracnose responsible for appreciable wastage of Jamaican bananas should be referred to as 'non-latent' or 'wound anthracnose'. Provided that fruit is marketed soon after turning from green to yellow, the 'latent' form of infection (Simmonds, 1941; Chakravarty, 1957) is of negligible commercial importance because it appears only on over-ripe fruit. Thus, latent infections were not considered in the current investigation.

Apparently healthy and uninjured green fruit was inoculated by making a small, superficial scratch in the peel and spraying with a suspension of *G. musarum* adjusted to approximately 2×10^4 spores/ml. Other details were identical with those for the experiments recorded in Table 4. The incidence of characteristic

Table 6. *Effect on anthracnose of Shirlan WS applied at intervals after inoculation: percentage numbers of lesions developed*

% Shirlan WS	Incubation period (hr.) 0	12	24	48	Mean
	Exp. A. Shirlan WS not washed off				
0	82	71	78	88	81·8
0·5	2	2	1	52	14·3
1·0	0	1	1	22	6·0
1·5	0	0	0	7	1·8
Mean	21·0	20·5	20·0	42·3	
	Exp. B. Shirlan WS washed off				
0	96	55	94	93	84·3
0·5	13	0	7	60	20·0
1·0	9	3	6	46	16·0
1·5	8	1	5	43	14·3
Mean	31·5	14·8	28·0	60·3	

Analysis of variance

Source	D.F.	M.S. Exp. A	Exp. B
Shirlan WS	3	566·28***	457·94***
Incubation	3	49·10***	146·84***
Interaction	9	9·64***	14·51***

*** Significant at 0·1 % level of probability.

lens-shaped lesions was recorded at full maturity and the results are summarized in Table 6.

Both sets of data indicate good control of *G. musarum* infection by Shirlan WS (Pl. 16, fig. 3). With incubation periods of up to 24 hr., there was very little difference between the degree of control in Exp. A and that in Exp. B. A significant and appreciable breakdown in control occurred after 48 hr. incubation: here, 1·5% Shirlan WS was more effective than lower concentrations, probably accounting for the significant mean squares for interaction of main effects. At all concentrations tested, control after 48 hr. incubation was better in Exp. A than in Exp. B. This may be due to removal, or at least considerable dilution, of fungicide during the water treatment. These experiments once more confirmed that Shirlan WS is not phytotoxic if fruit is washed after fungicidal treatment.

A final experiment compared effects on anthracnose of different periods of immersion in 1·5% Shirlan WS. The parallel experiment on finger-stalk rot showed that there were no significant differences between the variously treated control fruit. Thus in the present experiment, it was convenient to set up only eight control hands for each incubation period: these were immersed in water for 1 min. The results are given in Table 7.

TABLE 7. *Effect on anthracnose of Shirlan WS treatment followed by a water wash: percentage numbers of lesions developed* (*out of* 80)

Fungicide treatment	Incubation between inoculation and treatment (hr.)	Duration of treatment (min.) Momentary	0·5	1	2	Mean
1·5% Shirlan WS	0	23	9	2·5	1·3	8·9
	24	23	18	11	0	13·0
	48	73	54	69	43	59·8
	Mean	39·7	27·0	27·5	14·8	
None (control)	0	—	—	96	—	—
	24	—	—	91	—	—
	48	—	—	90	—	—
	Mean	—	—	92·3	—	

Analysis of variance (Shirlan WS data only)

Source	D.F.	M.S.
Incubation	2	258·58***
Immersion period	3	21·96***
Interaction	6	4·27*

* Significant at 5% level of probability; *** at 0·1% level.

It is evident that 1·5% Shirlan WS caused a marked reduction in disease incidence relative to that on untreated fruit. Percentage control showed a significant decrease between 24 and 48 hr. incubation ($P < 0{\cdot}001$). Immersion for 0·5 min. or longer in the fungicide was better than a momentary dip: 2 min. treatment gave significantly better control than shorter ones ($P < 0{\cdot}01$). The main effects were, therefore, similar to those in the parallel experiment on finger-stalk rot.

Main-stalk rot

Main-stalk rot was described by Wardlaw (1935). Although he distinguished clearly between this form of rot and finger-stalk rot, there is some confusion in the literature due to other workers referring to both rots as 'stem-end rot'. In this paper, Wardlaw's terminology is adopted.

Main-stalk rot on Jamaican bananas is caused chiefly by *G. musarum*, *Fusarium* spp. and, occasionally, by *Ceratocystis paradoxa* (Dade). These fungi readily colonize exposed tissues at the cut main-stalk ends. During the course of these and of earlier investigations (Meredith, 1960*a*) it was found that nystatin was not very effective against this disease; differences between the extent of *Gloeosporium* rot in treated and untreated stalks did not exceed 1–2 in. There was no apparent reduction when *Fusarium* spp. caused rot. Shirlan WS was more effective than nystatin, and 1 min. immersion in a 1·0 or 1·5% solution was sometimes sufficient to maintain the stalks in a healthy condition until maturity was reached.

Discussion

Although anthracnose and finger-stalk rot cause negligible loss due to inedibility, the unattractive appearance of infected fruit lowers its marketable value. Both forms of *Gloeosporium* rot develop from wound infections and it follows that, by very careful handling, disease could be prevented, rendering fungicidal treatment unnecessary.

Experiments described here indicate that Shirlan WS is very effective against *G. musarum* when the fungus is acting as a wound parasite. But the injurious effect of the compound on Lacatan bananas precludes its use in the manner described by Simmonds (1949). By washing fruit after Shirlan WS treatment, phytotoxicity was prevented and there was moderate and good control of finger-stalk rot and anthracnose, respectively. Histological studies have shown that, after 48 hr. incubation, *G. musarum* has traversed several cell layers (Meredith, 1960*a*). The fact that, after 48 hr. incubation, immersion for 1 min. in 1·0 or 1·5% Shirlan WS, followed by washing, brought about 25–50% control of anthracnose implies that the compound easily penetrates into wounds and can eradicate well-established infections. Earlier work on nystatin showed that there was no significant effect on anthracnose after 24 hr. incubation. Possibly the antibiotic, because of its insolubility in water (Olin Mathieson Chemical Corporation, 1958) is unable to reach relatively deep-seated infections. Some evidence to support this was provided by experiments in which it was found that percentage reduction of anthracnose decreased as the depth of scratch-inoculations increased (Meredith, 1960*a*). When finger-stalks are injured as described in the present paper, the resultant wound is much larger than the largest anthracnose inoculation tested. It was not surprising, therefore, to find little or no control of finger-stalk rot.

Rangaswami & Damodaran (1958) reported that nystatin had high *in vitro* activity against *G. musarum*. More recently Beccari & Golato (1959) apparently unaware of this work, confirmed this finding. The latter authors 'believe that a

dusting of stem-end cuts of banana bunches, before packing, should be successful to control stem-end rot, principally determined by *Gloeosporium musarum*, . . .'. Unfortunately no evidence from *in vivo* tests was presented to support this contention. Observations in the current investigation were unpromising as far as nystatin control of main-stalk (stem-end) rot was concerned, particularly when rotting was primarily of the *Fusarium* type. In small-scale experimental shipments, Castellani *et al.* (1958) also found that there was no control of *Fusarium* rot of the main-stalk.

In Jamaica, the severed ends of the main-stalk are pasted with a mixture of polyethylene polysulphide and mercapto-benzothiazole ('PEPS'): provided that there is good coverage of all freshly exposed tissues, excellent control of main-stalk rot is obtained (Meredith, 1959). It was noted that Shirlan WS reduced the extent of main-stalk rot, but this finding may have little significance under prevailing conditions, for after Shirlan WS treatment, 3–6 in. would be trimmed off each end of the main-stalk immediately before 'PEPS' treatment. Some additional control might be obtained if trimming were performed before dipping in Shirlan WS.

It remains to discuss some practical considerations that would arise if Shirlan WS is to be used commercially in Jamaica. Treatment can be carried out most conveniently at each of the five ship-loading wharves. It may be possible to treat some fruit in the plantation or at inland buying stations, but such fruit would constitute a minor proportion. Approximate estimates of the average numbers of bunches handled at each wharf per shipment are: Port Antonio, 60,000–100,000; Montego Bay, 40,000; Oracabessa, 50,000; Bowden, 35,000; Lucea, 6,000–10,000. In the plantations, either Bordeaux mixture or oil is used as spray material for the control of leaf spot (*Mycosphaerella musicola*). At the wharf, fruit showing Bordeaux spray residues is dipped in sodium bisulphate (22 lb./100 gal.) and then rinsed in fresh water. It would be a simple matter to interpolate a Shirlan WS tank between the acid and water tanks, the sequence of treatments therefore being acid—Shirlan WS —water—wrapping in Polythene tubing—trimming and applying 'PEPS' to main-stalk ends. In the case of oil-sprayed fruit, the acid wash is unnecessary; a slightly different arrangement would be required since this fruit is often wrapped in Polythene on arrival at the wharf. If one end of the Polythene tubing were left untied, the fruit could be dipped in Shirlan WS, allowed to drain off and then rinsed in water. The bag would then be secured and 'PEPS' applied.

Three main factors will probably contribute to the success of Shirlan WS treatment. First, it would be unwise to use concentrations lower than 1·0%, since 0·5% gave relatively poor control of *Gloeosporium* rot. Secondly, fruit should remain in the fungicide tank for at least 0·5 min. Finally, treatment should be carried out as soon as possible after harvesting. Arrangements are being made to test Shirlan WS in several commercial-scale experiments.

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EXPLANATION OF PLATE 16

Fig. 1. A 'hand' of green Lacatan bananas showing mechanically-induced creasing and associated blackening of finger-stalk tissues; photographed 1 hr. after wounding.

Fig. 2. Severe finger-stalk rot caused by *G. musarum*; all infections originated in wounded finger-stalk tissues.

Fig. 3. Effect of Shirlan WS treatment, followed by rinsing in water, on the development of anthracnose caused by *G. musarum*: Left: control fruit wound-inoculated and rinsed in water only. Right: similarly inoculated fruit treated with 1·0% Shirlan WS.

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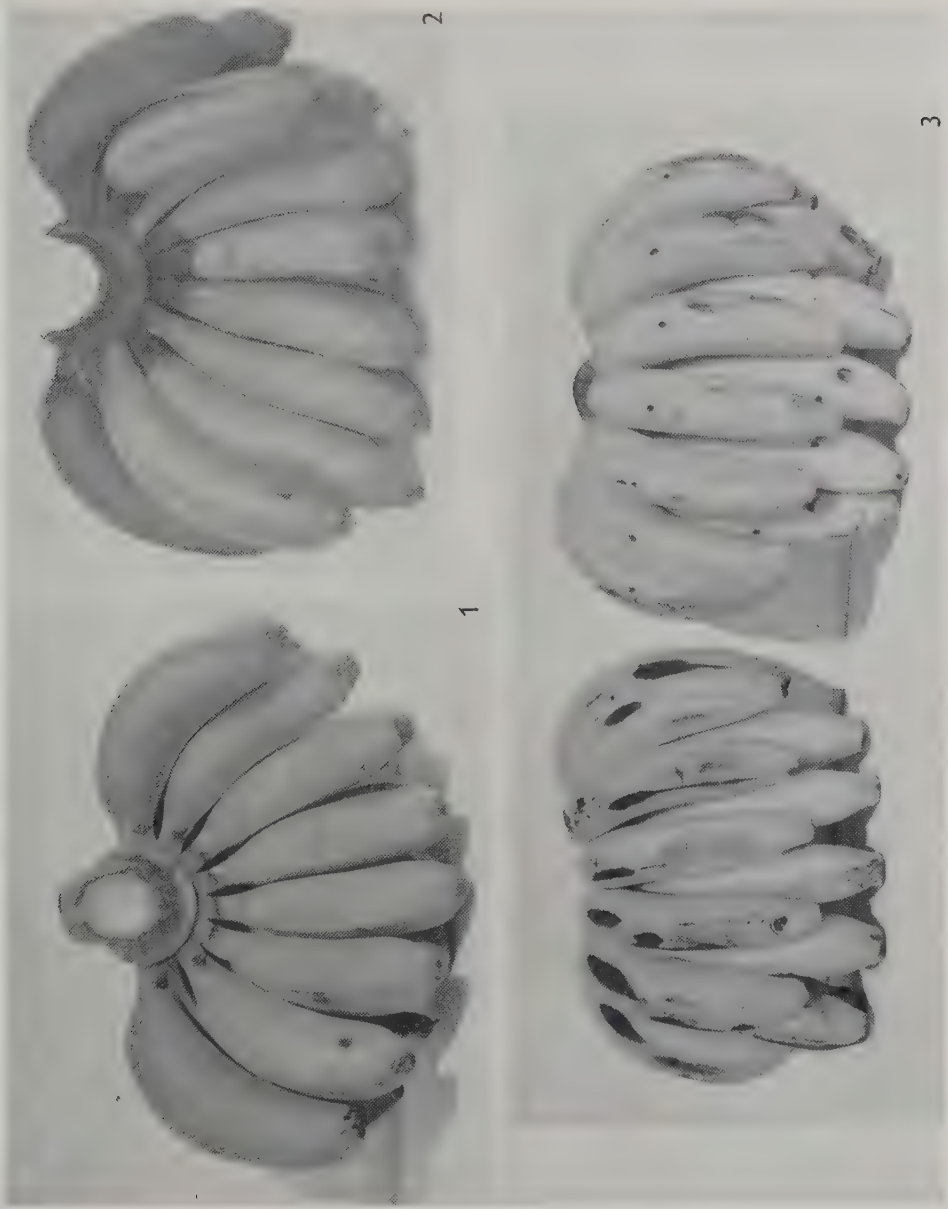

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PYRETHRUM CULTIVATION IN ENGLAND, 1925–37

By J. T. MARTIN

Long Ashton Research Station, University of Bristol

Small plot and field experiments on the cultivation of the insecticidal pyrethrum plant in England during 1925–37 showed that the plant grew well and produced satisfactory yields of flowers of high pyrethrin content: costings of the field trials, however, showed that the cultivation of the plant was uneconomic. Individual plants grown from seed differed considerably in growth habit and in the pyrethrin content of their flowers.

Introduction

Between 1928 and 1939, papers were published in the *Annals* recording the results of collaborative work between the Plant Pathology Laboratory of the Ministry of Agriculture and the Rothamsted Experimental Station on the cultivation of the insecticidal pyrethrum plant (*Chrysanthemum cinerariaefolium* Trev.) in England. Fryer, Tattersfield & Gimingham (1928) described the objectives of the work and experiments, using a bio-assay method, on the distribution of the toxic agent in the plant. Later accounts dealt with the relationship between the stage of development of the flowers and their pyrethrin content (Tattersfield, 1931), small plot and field manurial experiments (Martin & Tattersfield, 1934*a*; Martin, Mann & Tattersfield, 1939) and the effects of environmental conditions on flowering (Martin & Tattersfield, 1934*b*). The early work in England and the development of the Kenya industry were described by Tattersfield (1948) and West (1959), whose papers referred to other plot and field experiments, the details of which were not published. These details are now given with the object of completing the published record of the early work on pyrethrum. The late C. T. Gimingham of the Plant Pathology Laboratory was responsible, from 1927, for initiating the trials and recording yields and costings, and J. T. Martin, then at Rothamsted, from 1930 for the analytical work on the flowers.*

Small plot experiments

In the early 1920's pyrethrum, already established as a domestic insect powder, was finding increasing use in Europe in vine growing. Because of the unsatisfactory nature of the Dalmatian and Japanese supplies, the plant was grown in France and Switzerland: its successful cultivation as far north as Paris suggested to J. C. F. (later Sir John) Fryer, the Director of the Plant Pathology Laboratory, that it should be tried in England. In 1924 he obtained seed, of Swiss origin, from France, and in 1925 an experimental 1-rod plot was planted in the garden of the

* Some years ago, Gimingham handed to the writer the plot and field records with the hope that they and the analytical data would eventually be published.

laboratory at Harpenden. Seed was also obtained from Japan and Albania and additional 1-rod plots were set up in the garden in 1929 (for photographs of the plots see Tattersfield, 1948).

By 1928 Tattersfield had perfected his method for the determination of the pyrethrins I and II in the flowers (Tattersfield, Hobson & Gimingham, 1929). Earlier work by Tattersfield on the relationship between the degree of maturation of the flowers and pyrethrin content had been inconclusive. A bed of 108 plants (origin of seed not recorded) set out as seedlings in the garden in 1928, was therefore used by Tattersfield to examine this matter further. Analysis of the flowers of individual plants in 1929 showed that the pyrethrin content rose to a maximum in the fully open blooms (Tattersfield, 1931) and this was confirmed in 1930 (Martin & Tattersfield, 1931). All analyses from 1930 onwards were therefore made on the fully open flowers, which were separated from their stalks and allowed to dry on

TABLE 1. *Yields and pyrethrin contents of flowers from the small plot experiments*

Plot	Year of harvest	Equivalent yield (cwt./acre)	Pyrethrin I (%)	Pyrethrin II (%)
No. 17 Swiss strain seed.	1926	7·1*	—	—
Seedlings planted out	1927	3·9*	0·35†	0·35†
1925	1928	4·6*	0·5†	—
	1929	2·1*	0·55†	—
	1930	10·0	1·0	0·7
	1931	6·0	0·7	—
	1932	5·8	0·6	0·8
	1933	5·4	0·75	0·5
	1934	6·1	0·6	0·5
	1936	5·8	0·45	0·35
No. 22 Swiss strain seed.	1930	8·2	0·7	0·85
Seedlings planted out	1931	12·7	0·55	0·95
1929	1932	6·1	0·55	0·9
	1933	7·8	0·6	0·7
	1934	9·2	0·6	0·75
	1936	6·3	0·4	0·45
	1937	4·7	0·5	0·8
No. 23 Japanese strain	1930	8·9	0·7	0·7
seed. Seedlings planted	1931	10·7	0·55	0·8
out 1929	1932	5·5	0·5	0·9
	1933	6·8	0·55	0·6
	1934	9·3	0·5	0·6
	1936	7·5	—	—
	1937	5·2	0·5	0·65
No. 16 Albanian strain	1930	7·1	0·65	0·9
seed. Seedlings planted	1931	11·3	0·6	0·85
out 1929	1932	1·4	0·5	0·8
	Seed used			
Swiss strain	1930	—	0·4	0·65
Japanese strain	1930	—	0·4	—
Albanian strain	1930	—	0·4	—

* Immature flowers present.
† Analyses made in 1931.

trays, shielded from direct sunlight, in an unheated glasshouse. The pyrethrin contents were determined during the autumn and winter following harvest and were expressed on the air-dried flowers. A severe frost late in May, 1935, reduced the yields of flowers from all the experiments to insignificant amounts. Table 1 shows the results of these small plot trials.

Other small plot trials were started by the Plant Pathology Laboratory in 1926 at Wye and Swanley (Swiss and Japanese seed); Scilly Isles, Seale Hayne, Wisley and Worcester (Swiss seed) and East Malling and Sparsholt (Japanese seed). Experimental plantings were also made at Long Ashton, Kew, Lewes, Reading, Aberystwyth, Wisbech and Denham. The average yield of flowers from the plots derived from seed of Swiss origin was 5·5 cwt. per acre. The total pyrethrin contents of the flowers from the 1926 and 1927 crops, determined in 1928, did not exceed 1·1% (Tattersfield, Hobson & Gimingham, 1929; Tattersfield & Hobson, 1929), but immature flowers were present in the samples and some loss of activity had probably occurred in the interval before analysis.

Field experiments

Valuable information was obtained from the small plot trials on handling the plant and on its response to conditions in England. The data, however, were insufficient to assess the plant's economic possibilities. Field experiments in which costings were recorded were therefore arranged. Soils of different types were selected and plantings were made in 1929 at Long Melford in East Anglia, Wye and Swanley in Kent and Kemerton in Gloucestershire. Additional plots were set up in the following years at Long Melford and at two other centres, Thetford and Elveden, in East Anglia. The plots were of the order of ½–1 acre; the largest, sited at Long Melford (1931 planting), was 1¾ acres. Seedlings from seed obtained from France were raised in boxes in cool glasshouses and planted out in the autumn of 1929; the first flowers were obtained the following year. The plants were spaced 18 in. apart and the distance between rows varied from 18 to 24 in. After 1931, harvesting the flowers proved laborious, and at some centres, a combing method was adopted. Bunches of the flowers, held by the stalks, were pulled through a metal comb mounted on a wooden framework, the severed heads falling into sacking cloth. The flowers, dried on trays protected from sunlight, were stored in metal bins. The Wye experiment, including a photograph of the combing device, was described by Jary (1936).

The period of flowering, from the appearance of the small 'buttons' to the overblown condition, was about 8 weeks. Harvesting, when most of the flowers were fully open, invariably occurred during the first 10 days of July. A few more flowers formed later in the summer but never in sufficient numbers to warrant collection.

The soil types were as follows:

Long Melford. Medium loam of good texture and in good heart when the pyrethrum was planted.

Kemerton. Rather thin stony loam overlying limestone rock.
Wye. Chalky loam with flints.
Swanley. Light thin loam on chalk.
Elveden. Barren thin sand: typical 'breck' land.
Thetford. Light sandy soil, perhaps originally 'breck' land but long cultivated

TABLE 2. *Yields and pyrethrin contents of flowers from the field experiments*

Plot	Year of planting	Year of harvest	Yield (cwt./acre)	Pyrethrin I (%)	Pyrethrin II (%)
Long Melford	1929	1930	11·0	0·7	0·85
		1931	5·3	0·6	0·8
		1932	3·7	0·65	0·7
	1930	1932	5·7	0·55	0·55
		1933	3·8	0·55	0·5
	1931	1933	—	0·55	0·5
	1932	1933	—	0·45	0·4
Wye	1929	1930	3·2	0·85	—
		1931	12·7	0·55	—
		1932	5·6	0·7	0·75
		1933	6·1	0·6	0·45
		1934	6·7	0·65	0·65
		1935	1·6*	0·45	0·4
Swanley	1929	1930	4·2	1·1	1·05
		1931	4·7	0·7	0·85
		1932	3·0	0·55	0·8
Kemerton	1929	1930	4·3	1·05	1·0
Thetford	1930	1931	6·7	0·8	0·95
		1932	6·3	0·55	0·7
Elveden	1930	1931	1·7	0·5	—
		1932	2·0	0·45	0·65

* Late frost damage.

TABLE 3. *Costings in £ sterling of field experiments*

Plot	Year	Cost of cultivation per acre	Cost of harvesting per cwt. air-dry flowers	Cost of drying per cwt. air-dry flowers	Total expenditure per acre	Revenue from flowers per acre
Long Melford	1929	5·3	—	—	18·2	—
	1930	3·0	1·2	0·9	32·0	36·8
	1931	8·7	1·7	0·8	27·7	20·0
	1932	9·7	3·0	0·9	24·3	8·7
Wye	1929	16·1	—	—	36·4	—
	1930	10·5	0·8	2·3	22·4	12·0
	1931	11·4	1·6	0·4	39·5	44·6
	1932	10·3	2·4	0·5	29·8	21·0
Swanley	1929	3·0	—	—	21·4	—
	1930	4·2	2·3	0·7	19·5	13·5
	1931	3·2	2·1	0·6	16·4	19·5
	1932	2·5	3·6	1·1	18·5	12·0

and much more retentive than the Elveden soil. In good heart when the pyrethrum was planted.

Table 2 gives the yields and pyrethrin contents of the air-dried flowers from the field plots and Table 3 shows the costings of three of the field experiments.

Variation between individual plants raised from seed

The pyrethrin contents given in Tables 1 and 2 were determined on the mixed flowers from a considerable number of plants. In his work on the relationship between stage of development of the flower and pyrethrin content, Tattersfield (1931) also obtained valuable information on the degree of variation in the size and pyrethrin content of the open flowers from individual plants raised from seed (Table 4).

Table 4. *Variation in yield, average size and pyrethrin content of the flowers from individual plants raised from seed*

Plant	Yield (g.)	Aver. wt. of flower head (g.)	Pyrethrin I (%)	Pyrethrin II (%)
F 1	64·6	0·15	0·65	0·5
2	27·9	0·18	0·6	0·55
3	19·5	0·16	0·55	0·65
4	18·2	0·14	0·7	0·7
5	17·3	0·17	0·65	0·9
6	14·1	0·17	0·45	0·9
7	16·1	0·13	0·75	0·8
8	16·2	0·13	0·8	0·95
9	42·2	0·18	0·85	0·5
10	28·1	0·17	0·4	0·6
11	32·1	0·14	1·25	0·85
12	10·1	0·16	0·4	0·55
G 1	45·7	0·23	0·7	1·0
2		Plant died		
3	14·1	0·13	0·55	0·7
4	19·2	0·20	0·95	0·95
5	43·7	0·22	0·8	0·95
6	30·4	0·16	0·95	0·95
7	15·2	0·17	0·55	0·75
8	40·3	0·19	0·95	0·95
9	28·9	0·17	0·65	1·15
10	7·3	0·14	0·3	0·65
11	13·4	0·16	0·85	0·45
12	27·5	0·19	0·6	0·85

Plant growth habit and pyrethrin content of the flowers

In all the trials, the plants grown from seed showed considerable variations in morphological characteristics. Some plants produced long flower-stalks which tended to become interwoven and procumbent, making harvesting difficult; others bore short upright stalks carrying flowers, above the leafy growth, in a more or less horizontal plane. Fryer had in mind the possibility of cutting the flowers by machine; for this purpose, the plants bearing upright stalks were ideal. Work was

therefore done to see whether the pyrethrin content of the flowers and the plant's growth habit were correlated. Plants of six types selected on the basis of differences in morphological characteristics were divided into rooted shoots which were planted out in 1929. Analyses of the flowers obtained in the 3 following years revealed no useful relationship between plant habit and poison content of the flowers: this was confirmed in a similar experiment with plants grown at Wye.

Cross-pollination experiment

A cross-pollination experiment was begun in 1932 in an attempt to combine desirable plant characteristics with a high poison content of the flowers. Tattersfield's analyses of the flowers from the plants in his 1928 plot revealed two plants which produced blooms of high pyrethrin content but which showed widely different habits. One (plant F 11, Table 4) produced long flower stalks bearing

Table 5. *Pyrethrin contents of flowers from F 11 and G 4 plants*

		F 11		G 4	
	Year of harvest	Pyrethrin I (%)	Pyrethrin II (%)	Pyrethrin I (%)	Pyrethrin II (%)
Original plants	1929	1·25	0·85	0·95	0·95
	1930	1·1	1·0	1·05	1·05
Plants from subdivisions	1931	0·9	—	0·8	—
	1932	0·85	—	0·9	—
	1933	1·1	0·8	1·0	0·65
Original plants	1933	1·0	0·8	0·85	0·65

flowers with long petals; the other (plant G 4, Table 4), carried shorter, upright stalks and flowers with abbreviated petals. Selected flower heads were cross-pollinated (F 11 ♂ × G 4 ♀ and G 4 ♂ × F 11 ♀) and forty-eight plants from each crossing were grown in a bed of sixteen rows with six plants (*a–f*) in each row. Yield, average flower weight and average petal length were measured for 4 years. Because of other analytical commitments, pyrethrin content could be measured for only 2 years on the flowers from sixteen plants (eight from each crossing) selected at random.

The average weights of the flower heads from the original F 11 and G 4 plants were 0·14 and 0·20 g. and the average petal lengths 18 and 9·5 mm., respectively. The pyrethrin contents of the flowers from the parent plants and from plants grown from subdivisions of the parent plants over five years are shown in Table 5.

The results of the cross-pollination experiment for the years 1933 and 1934 are given in Table 6.

Discussion and conclusions

The pyrethrin values reported were determined by the 'long' acid method (Tattersfield *et al.* 1929) on 5 g. samples of flowers. The flowers used during the development of the method often contained less than 1·0% of total pyrethrins because of

the ages of the samples and the presence of varying proportions of immature heads, and of such flowers, 10 g. samples were needed for analysis. In the work reported, tests were made within a few months of harvesting and only fully open flowers were included. The high pyrethrin values then found necessitated modifications in the method, including decreasing the amount taken for analysis

TABLE 6. *Average weight, average petal length and pyrethrin content of flowers from the cross-pollination experiment*

	Plant	Yield (g.)	Aver. wt. of flower head (g.)	Aver. petal length (mm.)	Pyrethrin I (%)	Pyrethrin II (%)
			1933 harvest			
F11 female	1*e*	32·4	0·17	19	0·8	0·7
	2*e*	9·2	0·17	7·5	0·6	0·65
	5*c*	14·7	0·14	15	0·6	0·8
	6*e*	16·3	0·17	14·5	0·85	0·5
	9*f*	25·1	0·18	17	0·5	0·5
	10*a*	37·5	0·16	8	0·7	1·0
	13*d*	13·1	0·18	18	0·7	0·7
	14*d*	12·3	0·16	9·5	0·65	0·8
G4 female	3*b*	27·3	0·18	18	0·85	0·8
	4*a*	21·1	0·17	9	0·9	0·9
	7*a*	22·7	0·16	9	0·85	0·45
	8*e*	27·2	0·19	17·5	0·7	0·6
	11*d*	27·9	0·16	9·5	0·95	0·85
	12*e*	27·6	0·16	17	0·5	0·8
	15*f*	14·8	0·19	12	1·0	0·9
	16*c*	13·6	0·18	14	0·8	0·55
			1934 harvest			
F11 female	1*e*	61·0	0·17	19	0·65	0·5
	2*e*	13·1	0·16	6	0·75	0·4
	5*c*	16·5	0·15	14	0·7	0·7
	6*e*	29·8	0·15	15	0·8	0·3
	9*f*	51·8	0·18	16·5	0·45	0·4
	10*a*	10·5	0·16	7	0·65	0·75
	13*d*	40·0	0·17	16·5	0·7	0·6
	14*d*	39·6	0·19	10	0·65	0·6
G4 female	3*b*	48·7	0·15	18	0·7	0·65
	4*a*	48·7	0·19	9·5	0·85	0·7
	7*a*	33·2	0·18	9	0·85	0·35
	8*e*	34·9	0·16	15·5	0·75	0·4
	11*d*	9·8	0·19	10	1·05	0·75
	12*e*	33·3	0·19	15·5	0·55	0·75
	15*f*	35·2	0·19	10·5	0·7	0·65
	16*c*	19·7	0·14	13·5	0·85	0·45

(Martin & Tattersfield, 1931). The validity of the pyrethrin figures was checked in various ways. Many of the analyses were on 5·0 and 2·5 g. samples; the pyrethrin I values throughout agreed closely but rather higher results were obtained for pyrethrin II on 2·5 g. than on 5·0 g. samples. The exceptionally high values found on analysis of some of the samples were also checked by other analytical methods (Gnadinger & Corl, 1929; Seil, 1934; Wilcoxon, 1936) as these became available.

Satisfactory agreement on the level of pyrethrin content in the flowers was invariably obtained; for example, the rich Swanley flowers (1930 harvest) which showed 2·1% total pyrethrins by the acid method returned 2·0% (mean of five determinations) by the Gnadinger & Corl method. Widely differing levels of pyrethrin content found by analysis were also confirmed by bio-assay (Martin & Tattersfield, 1931). The pyrethrin I and II values on 5 g. samples could be reproduced to within 0·04; for inclusion in the tables the mean figures, as found, were therefore rounded off to the nearest 0·05, an indication of the order of significance that could be obtained.

The small plot experiments (Table 1) showed that pyrethrum plants producing high quality flowers could be grown satisfactorily in England. At the time of the work, the best samples of commercial pyrethrum on the market rarely contained more than 1% total pyrethrins. The first small plot set up (no. 17) produced crops of air-dried flowers equivalent to 5 cwt. or more per acre for 9 years. The pyrethrin content of the flowers, initially high, gradually declined as the bed aged. The plot was reluctantly discarded after 12 years of life, because of a severe weed infestation. Similar patterns of yields and pyrethrin contents of the flowers were seen in the other small plot trials.

These results were confirmed by the field experiments at the six outside centres (Table 2). Yields of the order of 4 or more cwt. per acre were obtained and the pyrethrin contents of the flowers, initially high, declined with time. The flowers from Swanley and Kemerton had exceptionally high pyrethrin contents (2% total). Although climatic conditions may have played a part in flower production, the general impression from many trials, including those at Long Melford and Wye, was that the highest yields were obtained within 2 years of setting out the plants. The costings of the field experiments recorded in Table 3 were of special interest. An average loss of £37 per acre was incurred in each of the experiments costed, the most expensive item being cultivation, particularly weeding, operations. Pyrethrum cultivation under the conditions prevailing at the time was clearly uneconomic, a conclusion reached with regret in view of the high quality of the flowers.

Tattersfield's results recorded in Table 4 showed that plants grown from seed differed appreciably in vigour and flower production, and that flower size and total pyrethrin content varied greatly. The average weight of the fully open flowers ranged from 0·13 to 0·22 g. and the total pyrethrin content from 0·95 to 2·1%. The analysis of the mixed flowers from a number of plants gave the impression that the pyrethrins occurred in approximately equal proportions; flowers from different individual plants, however, often contained pyrethrins I and II in different relative proportions. The flowers of the G 11 plant (Table 4) contained about twice as much pyrethrin I as II and those of the G 10 plant twice as much pyrethrin II as I.

Similar results were obtained in the analysis of the flowers from individual plants in the cross-pollination experiment (Table 6). Flowers of high quality (F 11, 2·1% and G4, 1·9% total pyrethrins) were crossed, yet individual plants

grown from the resulting seed gave in the following year flowers ranging in total pyrethrin content from 1·0 (plant 9*f*) to 1·9% (plant 15*f*). The ratios of pyrethrin I to II in the flowers again varied from 2:1 (plant 7*a*) to 1:1·6 (plant 12*e*). The petal lengths of the flowers from all the plants were examined in 1933, 1934 and 1936. In 1933, of the forty-eight plants derived from the F11 female plant, fifteen (31%) carried long-petalled flowers characteristic of F11, twenty-two (46%) bore short-petalled blooms typical of the G4 male parent, and the remainder of the plants (23%) showed flowers of intermediate petal length. The plants derived from the G4 female parent showed the same percentages of long, short and intermediate petal-length types (29, 46 and 25%, respectively). The petal-length characteristic of each plant in 1933 was maintained in the succeeding years. Jary (1936), who investigated the influence of the parent plant on the type of seedlings obtained following natural pollination in the field, found that approximately 70% of the plants raised from any one parent conformed to the parent type. Considering one plant characteristic, flower petal length, the results obtained in the cross-pollination experiment did not support this conclusion, for a preponderance of plants bearing short-petalled flowers came from the long-petalled F11 female parent. Of the sixteen plants examined for pyrethrin content, one (4*a*) stood out in possessing the characteristics required: it became a vigorous plant, producing about 50 g. of large-sized blooms in 1934, its short petal length was associated with a short-stemmed upright habit and the total pyrethrin content of the flowers was 1·55%.

The data for the parent plants recorded in Table 5 show that although there was some decline, high levels of total pyrethrin content of the flowers were maintained over 4 years. That individual plants produced flowers with high, medium or low contents of pyrethrins for a number of years was confirmed in other experiments. It was concluded that the pyrethrum plant could be improved by selection on vigour, growth habit and pyrethrin content, followed by vegetative propagation. A well-grown plant could be subdivided into many rooted shoots; one plant yielded ninety such 'splits' each of which, after growing-on in a cool glasshouse, came to maturity. In this way, the characteristics of the parent plant were retained, although a gradual decline in the pyrethrin content of the flowers had to be expected. Considering all the factors, including the progressive entanglement with weeds of the roots and leafy growth (see also Jary, 1936) the effective life of the plant was thought to be 5 years. The rich F11 plant was propagated in this way for many years to meet the overseas demand for seed. The descendants of some of the plants used in the experiments survive to this day.

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REVIEWS

Principles of General Physiology. Vol. II: *General Physiology.* By L. E. Bayliss. Pp. 848. London: Longmans, Green and Co. Ltd. 1960. 85*s.*

One man cannot cover adequately the whole field of physiology. Dr Bayliss has therefore concentrated on those aspects which interest him most, in this second volume of his revision of his father's book. These mainly comprise the physiological background to behaviour. There is a particularly good treatment of the physiology of nerve and nervous systems, and an unusual but welcome feature is the description of the principles of servo-mechanisms and their application to osmoregulation, gaseous exchange, temperature control and movement and posture.

Some emphases are open to criticism. Despite the exclusion of much of the usual mammalian physiology, a vertebrate bias remains. For example, it is stated that there are structural and physiological similarities between the mechanisms underlying ciliary and amoeboid movement and that of skeletal muscle; but the protozoan forms of locomotion are dismissed in a few paragraphs which are followed by a very full account of mammalian skeletal muscle, with no elucidation of these similarities. An odd inclusion is that of cell division and Mendelism, containing little of physiological interest.

The references are, not unreasonably, almost entirely to books and reviews. But there are some surprising omissions, such as Smith's review of the effects of cold on animals, de Bruyn's of amoeboid movement, Eccles's monograph on the physiology of nerve cells, and Hoyle's on the control of muscular contraction. These omissions are sometimes reflected in the text.

Nevertheless, this is a highly individual book which bears the imprint of a cultured and logical mind. The history of the subject is often referred to, and many useful outlines of experimental methods are given. Some of Sir William's 'frills' remain, such as Captain Cook on scurvy and Vergil on lupins, and for good measure Dr Bayliss has added Leonardo on water. The book can be recommended not only to the experimentalists and students of medicine and biology for whom it is intended, but also to the aspiring sixth former in search of a scholarship.

D. W. WOOD

Hackfruchtkrankheiten und Nematodenforschung. Editor H. Goffart. Mitteilungen no. 99 aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft. Pp. 119. Berlin-Dahlem: Paul Parey. 1960. DM. 13.50.

This volume commemorates the opening of a new building at the Institut für Hackfruchtkrankheiten und Nematodenforschung at Münster. Goffart, its director, briefly reviews its development over the past fifteen years, adding a list of its publications during that time. In a second article he deals with the history of plant nematology in Germany from Steinbuch in 1799 to the present day. The third article on the taxonomic value of the structural characteristics of *Heterodera* cysts discusses the various structures associated with the posterior ends of cysts and uses the German equivalents of Cooper's terminology. There are short diagnoses of eleven species, *H. avenae, H. cacti, H. carotae, H. cruciferae, H. galeopsidis, H. göttingiana, H. humuli, H. punctata, H. rostochiensis, H. schachtii* and *H. trifolii,* based mostly on cyst characteristics, but using some data about larvae. The paper is noteworthy for the many excellent photographs of the 'perineal' regions and of the patterns shown in the different species by the cuticle and subcuticle. Other articles deal with nematodes in vineyards; susceptibility of *H. rostochiensis* to radiation from a radium source; the effects of leaf loss and leaf regeneration on growth of sugar beet infected with virus

yellows; a virus disease of turnips transmitted by *Myzodes persicae* and called 'Vergilbungskrankheit der Stoppelrübe'; the effects of a systemic seed dressing 'Disyston' in controlling pests and the control of virus yellows in beet; and lastly the effects of artificial rain and spraying Metasystox on the development of aphid populations and the appearance of virus yellows.

J. B. GOODEY

Advances in Applied Microbiology. Vol. I. Editor W. W. UMBREIT. Pp. 304. New York: Academic Press; London: Academic Press (London) Ltd. 1959. $9.50.

Applied microbiology is the latest subject to be covered in the well-known '*Advances in*.....' series published by the Academic Press. The object is 'to provide the applied microbiologist with useful and up-to-date information', the stress being laid on the needs of the practical microbiologist.

Two of the reviews are mainly biochemical in nature—those by A. L. Demain on the mechanism of penicillin biosynthesis, and D. Perlman on the microbial synthesis of cobamides (vitamin B_{12} and related compounds). Contributions dealing with fermentation technology are presented by Herold and Nečásek on protected fermentation (the use of antimicrobial agents against contaminants), by S. Kinoshita on the production of amino acids (especially glutamic acid) by fermentation processes, and by Gerhardt and Bartlett on continuous industrial fermentation, including a detailed mathematical treatment of the subject.

Of more general interest to the average reader of the *Annals* are chapters on the status of antibiotics in plant disease control (D. Pramer), on insect microbiology (S. R. Dutky) and on the large-scale growth of higher fungi (Robinson and Davidson), a challenging subject where many hopes remain unfulfilled. The prospects of the preservation of foods and drugs by ionizing radiation are discussed by W. D. Bellamy, whilst E. O. Bennett brings together in a useful summary the factors affecting the antimicrobial activity of phenols. A. W. Phillips and J. E. Smith review germ-free animal techniques and their applications, of particular value in experimental pathology.

From the above list of subjects it is clear that primarily this is a volume for the specialist, and that few readers will be able to appreciate to the full more than two or three chapters. Equally, however, there will be few who will find no useful material in the book as a whole, and this first volume achieves its object of providing a wealth of useful information for the applied microbiologist, and, in particular, for the industrial microbiologist.

Minor criticisms are the over-predominance (80 %) of contributors from the U.S.A. and the surprising mis-citation of the name of Kluyver on three separate pages

R. J. W. BYRDE

Advances in Applied Microbiology. Vol. II. Editor W. W. UMBREIT. Pp. 384. New York: Academic Press; London: Academic Press (London) Ltd. 1960. $12.00.

It is natural to compare this book with volume I published last year, and also with volume 2 of *Progress in Industrial Microbiology*, the British publication. The two series appear to be running neck and neck. There is at least a steady if not an increasing flow of microbiological literature, not only in journals but also in books, and it is reassuring to the industrial microbiologist to find that the market is not being glutted with repetitive material, not that a certain amount of overlap is a bad thing. A glance at the chapter headings of these two volumes of the Series, and at the British Series, provides evidence that industrial microbiology is progressing and is not, as some non-biologically-minded people would seem to suggest 'on the way out'. In the sense of the utilization of micro-organisms as well as in the control of the predatory types (often the same organisms in a different environment) microbiologists look like being kept busy for quite a time yet. It appears, however, as if they will have to tear themselves away, far more than hitherto, from the Petri dish and the microscope ('platinum loop' stuff, as one student described it). They must go away and learn mathematics and statistical analysis. They must also woo engineers with a view to a marriage of

disciplines, the fruitful outcome of which will be (we hope) a brood of beautiful children called microbiological engineers. After all, microbiology in industry must face the economic facts, and although the biologist and the chartered accountant may be poles apart in outlook (the former, of course, is a *human* being), in the world to-day it rather looks as if the latter is the gentleman who indicates whether or not a new and improved process, be it biological or not, shall come to fruition or be nipped in the bud.

All microbiologists must be interested in at least one of the topics covered in this volume. The first chapter discusses Newer Aspects of Waste Treatment, which is a problem as vexed in this country as anywhere: it is surely necessary to cultivate the attitude that not only must we avoid as far as possible the excessive pollution of good water because it is very precious, but we should also utilize any waste if we possibly can. In so far as sewage is concerned we do indeed make use of the methane fermentation, but there are further possibilities, such as growth of algae for fodder, recovery of vitamin B_{12} and production of sulphur. With these constructive thoughts in mind, a later chapter, on the Biological Transformation of Solar Energy, is particularly recommended.

The chapter on Aerosol Samplers gives some idea of the complications arising in the study of these apparatuses. Impingers and impactors, selectors and discriminators, membrane and deep filters, instruments based on light-absorption and light-scattering, electrostatic devices and thermal precipitators are covered, with an extensive bibliography.

The Commentary on Microbiological Assaying is very interesting because the author is concerned not only with principles but with their practical application—down to dish-washing. In this context, however, it refers to the havoc which incompetent cleaning of glassware can play on sensitive assays. This chapter is the most readable one on this topic that we have met. We were shaken to learn that even with an elaborate automatic computer the precision of plate assays falls on Mondays, after holidays and 'after squabbles among the technicians'!

The increasing use of Membrane Filters as a matter of routine in many industrial laboratories makes the chapter on this topic useful; the technique is finding application in a widening field. The chapter following, on Microbial Control Methods in the Brewery, is very good as would be expected from an author of such repute. Those of us who are intimately concerned with this topic would like to see this section made compulsory reading in all breweries and bottling stores. If this reviewer may have a tiny dissentient squeak, it is that the name 'termobacteria' should now be dropped. It has no place in determinative bacteriology and the fact that it is listed in Bergey as a synonym for *Acetobacter aceti* adds further confusion!

Newer Developments in Vinegar Manufacture is an apposite chapter. There used to be much 'hush-hush' about this, some of which one suspects was associated not so much with the concealment of technical 'know-how' as with concealment of primitive procedure. Now, with International Vinegar Congresses, the open discussion of submerged fermentation and the elaboration of suitable equipment, the situation is changing. An ancient fermentation process has learnt from antibiotic industry. Even brewers now have to show an interest in continuous fermentation.

The Transformation of Steroids by Micro-organisms is being increasingly written about, not only because of its scientific interest but because of its economic importance as has already been shown in one or two directions. A chapter on the topic is therefore appropriate here and it shows how similar are microbial steroid hydroxylations to mammalian, with perhaps rather greater versatility on the part of the micro-organisms.

The last quarter of the book is given over to eight papers comprising a Symposium on Engineering Advances in Fermentation Practice held in New Jersey in 1959. The contents cannot be adequately discussed here, but they deal with vital aspects such as scaling-up of fermentations, air and media sterilization, kinetics and continuous fermentation.

H. J. BUNKER

Der Vielfrass (Gulo gulo *L.* 1758). *Zur Kenntnis seiner Naturgeschichte und seiner Bedeutung für den Menschen.* By P. KROTT. Monog. der Wildsäugetiere, no. 13, pp. 1–159 with 43 photographs, 7 tables, and 5 maps. Göttingen: Institut für Jagdkunde der Universität. 1960. DM. 20·75.

The author has produced a remarkable monograph of an animal which is both difficult to observe and thinly distributed. From a preliminary survey in ideal wolverine habitats in Finland and Sweden, the author realized the impossibility of regular and detailed study by this means alone. He therefore reared young wolverines and observed their growth, development and behaviour. Next, he released hand-reared animals in good wolverine country in the hope that he would see more of them than of truly wild individuals. In the event, he had considerable success and was able, for example, to plot the territories of his animals and the points of contact between them and wild wolverines.

About 80 % of the book consists of the author's own observations; the remainder includes sections on systematics, distribution, capture by trappers and the wolverine in history. The monograph is very well illustrated with photographs by the author and others. J. D. LOCKIE

Control of Rats in Sewers. By E. W. BENTLEY. Ministry of Agriculture, Fisheries and Food, Technical Bulletin, no. 10, 22 pages, 4 plates. London: H.M.S.O. 1960. 2*s.* 6*d.*

The control of rats in sewers has been recognized as basic to all rat control in urban areas since the 1919 Act. No scientific approach to the problem was made, however, until the Second World War when the work of Chitty was applied vigorously to most of the conurbations in Great Britain. The comparative success of these treatments, particularly in relieving surface infestations, has led to continued research into the biology of the rat in sewers. Dr Bentley is to be congratulated on producing a vade-mecum for Local Authorities, in which he gives not only excellent rule-of-thumb instructions but also the reasons for them. From the beginning of this work ideas of considerable practical value have continued to come from inspectors of local authorities, and it is pleasing to see so many incorporated in the text. Dr Bentley gives not only the biological and research background to the work but very rightly emphasizes the necessity of adequate planning and the rejection of false economy in operations. It is here that the local man can contribute so much, and this Bulletin will enable him to do so intelligently. H. R. HEWER

Introduction to Probability and Statistics. By H. L. ALDER and E. B. ROESSLER. Pp. 252. San Francisco and London: W. H. Freeman and Co. 1960. 20*s.*

This book is intended to form the basis for a one-semester (45-hour) course in probability and statistics. In addition to describing the simplest statistical notions and techniques, there are short chapters on index numbers and time series. The book is attractively produced and well indexed and the price is very reasonable.

In view of this, the contents are a great disappointment. Some nine-tenths of the material might have been written thirty years ago and would have been open to criticism even then. The order of presentation is dully familiar—measures of central tendency (mean, median, mode, geometric mean, harmonic mean); measures of dispersion (range, mean deviation, variance and standard deviation, the last with the sample size divisor rather than the degrees of freedom); the Normal distribution introduced as a limiting form of the binomial; separate chapters on 'large' and 'small' sample methods of hypothesis testing. The analysis of variance is omitted as being too advanced, instead of being used to unify all the common techniques for handling measured data.

So many elementary texts in statistics have been published recently that any new one has stiff competition to meet. This book is well down among the also-rans. M. J. R. HEALY

Methods of Testing Chemicals on Insects. Vol. II. Edited by H. H. SHEPARD. Pp. 248, photolithographed. Minneapolis: Burgess Publishing Co. 1960. $5.00.

The publication of the second volume of this series follows commendably closely on that of the first and many of the contributions are more up to date than those in the earlier volume [*Ann. appl. Biol.* **47**, 184]. There continues to be, however, a great diversity in the style, length, critical approach, and useful content of the various chapters, the standard of two or three of which could well have been raised by more editorial guidance. One wonders also what happened to the editorial pencil when checking some parts of the text, especially the paragraph on p. 12 which contains two seemingly contradictory statements about some of the editor's own published work.

The purpose of this second volume is to discuss factors affecting experimental results, and methods for screening candidate insecticides for specific purposes. The first three chapters deal respectively with the effects of different conditions before, during, and after exposure, subjects already treated in part in the first volume. The ten chapters following describe in varying extent of detail tests concerned with treated foods, space sprays, soil insecticides, fabric pests, repellents, package penetration, attractants, acaricides, and veterinary preparations (external and systemic). Sixteen of the authors hail from the U.S.A. and the seventeenth from Canada; and the 401 references from North American sources, out of a total of 500, indicate that emphasis is laid on the American viewpoint.

The index is better than that in vol. I, but could justifiably be rather more detailed in a book suited to reference rather than to continuous reading.

E. A. PARKIN

The Biology of Marine Animals. By J. A. COLIN NICOL. Pp. 707. London: Pitman. 1960. 95*s*.

The sea as a natural environment imposes a set of well-defined conditions upon its inhabitants, and it may therefore be considered justifiable to assume that all marine animals have a certain number of adaptive characteristics in common. Dr J. A. Nicol's book focuses interest on the comparative physiology of marine animals, with limited excursions into the fields of behaviour and ecology. As all major animal groups, with the notable exception of the insects, are represented in the sea, this book, quite naturally, assumes the status of a Treatise on Comparative Physiology.

Were it not for the rather prohibitive price, I should say without hesitation that this book could go a long way to remedy the regrettable shortage of palatable students' textbooks in Comparative Physiology. It is lucidly written, excellently illustrated and, above all, most meticulously accurate. On the whole the text is not overburdened with quantitative data, as these are set out in separate tables which furnish valuable information to the research worker.

The author's wide experience both as a marine zoologist and as an experimenter, has enabled him to present just the right kind of synthesis between the particular aspects of the marine environment and the great generalizations of functional biology.

Five chapters on water balance, ionic regulation, respiration, feeding, digestion, and excretion are followed by six chapters on receptor and effector systems, including pigmentary effectors and organs of luminescence. Animal associations and defence mechanisms are dealt with in two further chapters and there is a useful appendix on saline media. All chapters are followed by up-to-date lists of references and there is a comprehensive subject index.

The book can be warmly recommended to all biologists.

O. LOWENSTEIN

The Effects of Pollution on Living Material. Edited by W. B. YAPP. Symposia of the Institute of Biology, no. 8. Pp. 154. London: The Institute of Biology. 1959. 25*s*.

This book contains papers read at the Institute of Biology Symposium in 1958 and deals with water pollution, air pollution, and the disposal of radioactive wastes. Papers by F. T. K. Pentelow, M. B. N. Hynes and Prof. G. E. Newell provide synoptic reviews of the effect of pollution on fisheries, stream biology and the ecology of estuaries, while three more papers give detailed accounts of research into the modification of the chemical condition of polluted waters by the activities of living organisms. Despite the merit of the individual papers, this has resulted in unequal coverage of the subject of biology and water pollution, which should have been corrected by including recent work on the levels of chemical and physical conditions which aquatic organisms can tolerate; such studies are an essential tool when controlling pollution to preserve plants and animals of economic, recreational or aesthetic importance.

Four papers on air pollution cover their subject more evenly. The first paper (Williams) reviews the sources of air pollution and the chemical, physical and meteorological background, while Bleasdale and Lawther describe its effects on plant growth and on man. Ruth Allcroft deals with the more restricted topic of fluorosis in farm animals.

R. H. Burns gives an account of the methods used to dispose of radioactive wastes. A special problem here is that living organisms can concentrate some radioisotopes to a level at which they may be dangerous, and two further papers describe research on this topic. In discussing the genetical effects of radiation, T. C. Carter points out that the effects of the levels of radiation to which the population at large is exposed are assessed by extrapolation from observations made at much higher doses, and pleads for extensive research on the dose/mutation relation in the lower dose range; it seems that over the whole field of pollution more research is needed to determine the conditions to which living organisms can be subjected without harm.

D. W. M. HERBERT

Taxonomy of Flowering Plants. By C. L. PORTER. Pp. xii + 452. San Francisco and London: W. H. Freeman and Co. 1959. 44*s*.

This attractively produced book consists of an elementary but well-documented treatment of the history, principles, and methods of angiosperm taxonomy, together with illustrated descriptions of just over a hundred families representative of the North American flora. It is intended as a textbook, particularly for 'undergraduate students in such practical fields of study as agronomy, range management, forestry, and wildlife management and conservation'. Emphasis is on the recognition of families. Most of the smaller families are dealt with conveniently at a single page opening, the text on one side facing a page of illustrations on the other. The more extended treatment of some of the larger families, as the Cyperaceae, Poaceae (Gramineae), Fabaceae (Leguminosae) and Asteraceae (Compositae), includes keys to the subfamilies or tribes; by comparison, the treatment of the Brassicaceae (Cruciferae) and Apiaceae (Umbelliferae) seems rather meagre. The illustrations consist of numerous line drawings (by E. L. Gillespie), with about seventy photographs and a coloured frontispiece. The floral diagrams, on which much stress is laid by the author, are on the whole disappointing: many are badly proportioned or unnecessarily complicated, and the representation of sepals and petals is the reverse of that usually employed. Otherwise, the illustrations are excellent. The book is well indexed and there is a useful glossary. The revival of the term 'phytography' (chapter 8) for the art of plant description is commendable; but 'bostryx', 'cincinnus', 'rhipidium', and 'drepanium' are terms that would have been better omitted. Based on the author's lecture outlines over a period of some 25 years, this textbook may be safely recommended not only for the classes of student to whom it is particularly addressed, but also for those studying botany as part of a general science degree, and for teachers in schools.

T. E. T. BOND

Bananas. By N. W. Simmonds. Pp. 466. London: Longmans, Green and Co. Ltd. 1959. 45*s*.

The publishers of this book are to be congratulated on their choice of author. Only someone intimately acquainted with the Australimusa and Eumusa Series of banana clones is fully qualified to describe the botany of the banana plant. N. W. Simmonds believes that the most primitive edible bananas originated and are still to be found in south-east Asia, mostly as diploid strains of *Musa acuminata* and, to a lesser extent, of *M. balbisiana*. Subsequent hybridization resulted in the formation of diploid, triploid and tetraploid clones which gradually spread in varying degrees throughout the tropics and subtropics where they became known by a perplexing number of vernacular names. Possibly the most valuable part of the book is the masterly manner in which order has been established out of this confusion. The difference between 'bananas' and 'plantains' is at last clearly explained.

Several chapters are devoted to the presentation of an extensive collection of facts concerned with the growing, harvesting, transport, ripening and trading of bananas. The subject-matter has been obtained not only from literature, but also from personal contacts made and experience gained during the author's world-wide travels. The chapters on major pests and diseases contain many technical details which seem somewhat out of proportion to the rest of the book. This, however, is understandable when it is realized that all banana-breeding research has so far been founded on the need to attain resistance to disease, primarily Panama Disease, *Fusarium oxysporum* f. *cubense*. The comparative brevity of the chapter on banana breeding is doubtless due to the author's modesty over his own outstanding achievements in this specialized field of research.

A few inaccuracies have inevitably crept into the text; for instance, ascospores of *Mycosphaerella musicola* cannot be liberated from dry leaf spots, an important factor in the aetiology of Sigatoka. The large number of excellent illustrations perforce contribute to the unfortunately high price of the book but their inclusion has been well worth while. R. LEACH

Atlas of Bacterial Flagellation. By Einar Leifson. Pp. 171. Academic Press Inc. New York and London. 1960. $7.00 (?£2. 10*s*. 0*d*.).

Einar Leifson, the deviser of the well-known method of staining the flagella of bacteria, has produced a fascinating book of photographic reproductions 'showing the shape and arrangement of the flagella on representative strains of all available species of bacteria'. The staining method is given in full, together with useful comments on the importance of the concentration of the bacterial suspension and the pH and the temperature of both mordant and stain. The general practice is to make up the tannic acid mordant fresh each time but Leifson points out that if kept in the refrigerator it will last for one to two months and in the 'deep freeze' indefinitely.

The flagella are sensitive to pH and in some bacteria, for example, *Proteus* grown in a medium containing monobasic potassium phosphate (acid) appear 'curly', i.e. with short wavelength, while if the medium contains dibasic potassium phosphate (alkaline) the flagella appear 'normal', i.e. have a normal long wavelength.

Strains and species of fifty-seven genera are illustrated together with notes on the genera and the characteristics of the flagella.

It may be a little disconcerting to realize that authentic stock cultures may consist of one or more types of these characters, for example, differences in wavelength of the flagella even on the same cell. Some appear to have straight as well as 'curly' (wavy) flagella and these strains can be separated out by plating and they remain constant. Hitherto one had thought that these differences were due to one's own idiosyncrasy in staining until Prof. Pijper's discovery in 1954. These observations have led Leifson to subdivide the generally accepted classification of flagella arrangement into *polar*, *subpolar* (*a*) monotrichous, (*b*) multitrichous; *lateral* (*a*) monotrichous, (*b*) multitrichous; or *mixed* 'two or more flagella of distinctly different appearance in different locations'. On account of such observations

Leifson has questioned the systematic position of *Chromobacterium* placed by Bergey along with *Agrobacterium* and *Rhizobium*. The reviewer entirely agrees with Leifson though on different grounds.

In the reviewer's experience Leifson's method gives beautiful results perhaps owing to the contrast between red flagella and blue bodies—a contrast which is not apparent in the illustrations of the Atlas—but for photographic reproduction the 'silver' method is not to be surpassed, for example, those published by Migula in 1900.

Leifson assumes with most bacteriologists that the 'flagella are undoubtedly the locomotor organs of bacteria', but the opposite view held by a few, notably Prof. Pijper and the reviewer, receives a certain amount of support by the many references in Leifson's text to the effect that 'bacteria with the curly (short wave) type of flagella are sometimes poorly motile, and those with straight flagella are comparatively non-motile' (p. 2). Again 'organisms with straight flagella are either non-motile or show only a non-progressive spin or wiggle' (p. 15). Similar statements occur on pp. 104 and 112. This is not the place to elaborate one's reasons for considering that flagella (?appendages) are *not* the organs of locomotion. Suffice it to say that in the reviewer's opinion the 'dog wags the tail' not the tail wags the dog!

Leifson's Atlas will be of great interest to most bacteriologists although many of them no longer rely entirely upon the arrangement of the flagella for generic diagnosis, which now involves colony characters and biochemical activities. The Atlas may also stimulate further research into the nature of the mechanism responsible for the motion of flagella.

W. J. DOWSON

Diagnostik der Bakterien und Actinomyceten. By N. A. KRASSILNIKOV, Moscow, translated into German by R. W. WITTWER and R. DICKSCHEIT. Pp. 813. Gustav Fischer, Jena, 1959. 59 DM (£5).

The original Russian text of this voluminous work first published in 1949 has now been translated by two German bacteriologists without any reference to more modern works. The classification and nomenclature are chiefly those of Krassilnikov but also partly of Bergey, 1930. This may be confusing to those European and American bacteriologists who care to study the book but are presumably acquainted with Bergey, 1947, 1958; or as far as the Actinomycetes alone are concerned, with C. Stapp's recent monumental volume, *Bakteriosen einschliesslich Streptomykosen*, reviewed in this *Journal*, vol. **45**, p. 237, 1957.

The book is well printed and contains numerous original illustrations very well reproduced. It should form an interesting addition to the History of Science Series as well as illustrating how Russian bacteriologists differ from others in regarding the taxonomy of these organisms.

W. J. DOWSON

A Review of the Biological Control of Insects and Weeds in Australia and Australian New Guinea. By FRANK WILSON. Pp. 102. Farnham Royal: Commonwealth Agricultural Bureaux. 1960. 25*s*.

Other excellent histories of biological control have been written but the present book is the first complete account of such work in Australia. It was well worth writing and is well written.

The first question always put regarding attempts at biological control is, Has it worked? Mr Wilson answers this admirably in two summaries, which all should read, it certainly having worked in many cases but not in others. The second question that should be put is, Why has it worked, or not? The author gives many of the answers under the individual projects and in an interesting Discussion, in which he considers various aspects of biological control in Australia. It is in the main text that points for further thought occur and this makes fascinating reading. It is a factual account of the attempted control, species by

species, of pest insects (almost all of plants) and of pest plants. To import the 340 or so beneficial species from some 42 countries was only the first problem, other well-known problems connected with biological control being mentioned. As the author rightly says on p. 77, liberation of the beneficial species in the new country alone provides the evidence of capacity or incapacity to control the pest. Even so the number of mis-importations and non-releases does appear to have been high. Of releases, mention is made here and there of the numbers involved but there is no general assessment of the relation of numbers, in one release or in waves, to the results. Although the value of selected races in biological control is now receiving attention references to races or strains are only loosely given in the present work, but it could well serve as a source-book for ideas to this end, particularly concerning the evolution of a species when transferred to an entirely new country and environment. There is a good index of species; but an index to subjects, countries, technical problems, etc., would have been an advantage. The words of Dr Simmonds in the Foreword can well be echoed; 'It is hoped that similar reviews for other parts of the Commonwealth will follow in due course.'

E. B. BASDEN

The Ecology of Soil Fungi. Edited by D. PARKINSON and J. S. WAID. (An International Symposium). Pp. xii + 324. Liverpool University Press. 1960. *42s. 6d.*

This book is the outcome of the International Symposium held in the Botany Department of Liverpool University in 1958. It is divided into six sections, each headed by a review paper: Methods for isolation and estimation of activity of fungi in soil; The growth of fungi in soil; Antagonisms in soil; Dynamic equilibria of soil populations; Problems associated with the decomposition of organic matter; and Physiology of soil fungi. Succeeding each review are from three to five papers, mostly (but not all) relevant to the theme of the section, and each section concludes with a report of the discussion which terminated each session of the Conference.

Much attention has been paid to the differentiation of the activity of fungi in soil from their mere presence as dormant spores. Several contributions report on fungi colonizing diverse microhabitats in various soils; pine needles, couch grass debris, cellulose, chitin and keratin, roots and root zones (rhizospheres) of several plants. The occurrence of fungi in the specialized environments of desert sands and tidal mud flats is reported. Contributions deal with the phenomenon of fungistasis (that widespread inhibition of germination of fungus spores characteristic of soils); with the influence of the rhizosphere on this phenomenon, and of the rhizosphere on the bacterial flora of soils. Decomposition of the more resistant forms of soil organic matter, of humic acid, lignin and lignin-related molecules, is studied and their special problems critically discussed.

The ecology of micro-organisms, and the biological and biochemical factors which underlie it, is a discipline which is in a state of active development. This book was intended to further the development with reference to soil fungi. With an emphasis on approaches and ideas, I think it fairly fulfils this aim. It is unfortunate that two years separated the Conference from the publication. The book is well produced and complete with indexes to authors, subjects and micro-organisms.

H. T. TRIBE

Introduction to Plant Geography and Some Related Sciences. By NICHOLAS POLUNIN. Pp. xx + 640; folding map and 184 figs. London: Longmans, Green and Co. Ltd. 1960. *60s.*

Plant geography is here interpreted by Prof. Polunin as including not only all the geographical manifestations of plants both singly (as individual species) and collectively (as vegetation), but also the reasons behind these manifestations. Thus, about a third of the book deals with the 'related sciences' of plant morphology and classification, ecology, and economic botany; to all of which, in the author's words, 'in some modest degree it may also

serve as a general introduction'. It is questionable whether this extended interpretation will commend itself to the professional botanist; but it will be welcomed by the layman, and by the geographer and others whose knowledge of botany may be limited. Bearing in mind its wide scope, as indicated correctly by the title, the volume under review is to be regarded as of quite outstanding merit.

There are eighteen chapters, each concluding with a book list for further reading, usefully annotated. *The Journal of Ecology* and other periodicals are noted as sources of further information for the specialist but, wisely in the reviewer's opinion, no individual articles are cited. After the introductory chapter, in which the author's aims are stated and the plan of the book briefly described, there are four chapters devoted respectively to an introductory survey of the various groups of plants, their autecology and life forms, their dispersal and migration, and their evolutionary development. Chapters VI and VII deal with the facts and underlying theories of the past and present distribution of plant species, and it is here that the author really comes to grips with his subject, dealing in masterly fashion with such controversial issues as the 'nunatak' hypothesis, continental drift, the geographical effects of polyploidy, and the interpretation of the major types of discontinuity of range. This consideration of 'natural' distributions is followed by a chapter on man-made ones, both of crops and weeds (and of some plant diseases). The intention here is excellent and the result most valuable; but the same cannot be said of the next chapter, entitled 'Vital Importance to Mankind', which is virtually a mere catalogue of the world's useful (and harmful) plants and plant products and as such could well be omitted from any further edition, retaining only the book list which would supplement the references to chapter VIII. The remainder of the book is concerned with the distribution of vegetation as distinct from that of the individual species of plants. Chapter X deals with the environmental factors controlling the development of vegetation and chapter XI with the main habitats and with the concepts of succession and the climax. The next five chapters, comprising about one-third of the whole work, deal with the vegetational types respectively of temperate and adjacent lands, of polar lands and high altitudes, of tropical and adjacent lands, of fresh and inland saline waters, and of seas. In these, the treatment is straightforward and readable, being enlivened by many details from the author's personal experience and other sources of first-hand information. The two final chapters revert to the study of landscape and to the practical applications of plant geography in a world where modern man is the 'biotic superdominant'.

The book is well and attractively produced and illustrated, and is not expensive. Fewer than a dozen misprints have been noted. One sentence, discussing the flora of New Zealand in relation to the hypothesis of continental drift (p. 167), ends obscurely; and, of the many excellent and informative maps, only one (fig. 45B) is at all difficult to follow, on account of the smallness of the lettering. The index, which serves also in place of a glossary of technical terms, appears to have been very carefully compiled. Among the references and notes for further reading, L. Dudley Stamp's '*The Land of Britain*...' (2nd edition, 1950) should have been included in chapter XVII; and J. C. Willis's invaluable '*Dictionary*...' in chapter IX, if not elsewhere. It is a pity also that the latter author is not mentioned by name, in connexion with his hypothesis of 'Age and Area', in chapter VII.

This volume deserves to be read by a very wide public. For students of geography, agriculture, botany and the cognate sciences, and for their teachers, it will undoubtedly become the standard introductory work on the subject in the English language.

T. E. T. BOND

THE ANNALS OF APPLIED BIOLOGY

The Annals of Applied Biology is conducted by the Association of Applied Biologists and is published by the Cambridge University Press. The Association exists to further the study of all aspects of Biology and to correlate pure science with practice. Meetings are held about six times a year for informal discussion and the reading of papers.

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